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Voluntary Food Intake of Pregnant and Non-pregnant Red Deer Hinds During the Gestating Period

A thesis submitted for the degree of
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by
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**Voluntary Food Intake of Pregnant and
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by

I.C. Scott

Efficient venison production systems rely in New Zealand on maximal growth of deer calves before their first winter. This is facilitated by earlier calving (i.e. March conception for October/November calving). However, previous research has shown that hinds conceiving early in the breeding season have a longer gestation length than those conceiving later, negating some of the gains of achieving early conception. It is hypothesised that a moderate energy imbalance during the last trimester of pregnancy influences fetal growth trajectory resulting in variation of gestation length. While young red deer, adult stags and non-pregnant hinds exhibit a photoperiod-mediated reduction in voluntary food intake (VFI) during winter, it is not known if this occurs in pregnant hinds. Such a decrease in VFI would exacerbate a moderate energy imbalance during the third trimester of pregnancy. The objective of this study was to test the hypothesis that pregnancy status does not affect the photoperiod-mediated reduction in VFI of red deer hinds during winter. Seven pregnant (P) and seven non-pregnant (NP) hinds were housed indoors in individual pens from April to November where they were offered daily an *ad libitum* pelleted ration. Food intake was measured daily and hinds were weighed and body condition scored (BCS) fortnightly.

In addition, blood samples were collected at 4-week intervals to determine plasma concentrations of two appetite regulating hormones, leptin and ghrelin. Mean (\pm SEM) live weight (LW) on 27 April was 117.1 ± 6.0 kg and 124.1 ± 10.3 kg for P and NP hinds, respectively. On average, P hinds gained 74 g/day and NP hinds lost 27 g/day ($P < 0.05$) in autumn. Mean live weight (LW) of both groups then increased for the remainder of the study with no significant difference between groups during specific time periods. Body condition score (BCS) of both groups increased during autumn and winter ($P > 0.05$), but whereas BCS of P hinds decreased, that of NP hinds increased in spring (-0.001 BCS units/day vs. 0.006 BCS units/day; $P < 0.05$). Pregnancy status of the hinds had no significant effect on mean VFI throughout the trial except for the last five days before parturition when VFI of P hinds decreased dramatically ($P < 0.001$). Mean VFI of both groups was significantly higher about 1 May and 1 November than 1 July (0.72 ± 0.05 , 0.69 ± 0.03 and 0.58 ± 0.05 MJME/kg LW respectively; $P < 0.05$). There was a significant negative correlation of gestation length with mean VFI during the study period ($P < 0.05$), such that, for every 0.1 MJME/kg LW^{0.75}/day increase in mean VFI, gestation length decreased by 6.4 days. Pregnancy status had no significant effect on plasma concentration of leptin or ghrelin at any of the sampling times ($P > 0.05$). Circulating leptin concentration was associated significantly ($P < 0.01$) with BCS, but not VFI. Results from this study support the hypothesis that pregnancy status does not affect the photoperiod-mediated depression in VFI of red deer hinds during winter. It is suggested that to mitigate effects of the observed moderate energy imbalance during the last third of pregnancy, farmers should ensure hinds are in good condition going in to winter and that high quality feed is available throughout gestation.

Keywords: red deer; adult; hinds; non-pregnant; pregnant; voluntary food intake; gestation length; day length; live weight; body condition; leptin; ghrelin

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Chapter 1: Introduction

1.1 The problem stated

Efficient venison production relies on maximal growth of the red deer calf before its first winter (birth – 6 months of age). To this end, significant research effort has been expended to advance the calving date of red deer hinds to better align the nutritional demands of a lactating hind with pasture growth and quality under New Zealand lowland farming conditions (see review: Asher *et al.*, 1996). However, it would appear that evolution has ensured robust compensatory mechanisms are in place to counter such attempts to advance calving date.

Recent research has shown that hinds conceiving early in the breeding season have a longer gestation length than those conceiving late, and conversely, those conceiving late in the breeding season have a shorter gestation length (Scott *et al.*, 2008a). Also, Asher *et al.* (2005a) reported that a moderate energy intake imbalance during the last trimester of pregnancy in red deer was compensated for by varying gestation length to ensure optimal birth weight at the time of parturition.

It is well documented that red deer have a photoperiod-mediated reduction in their food intake during ‘short days’ (Pollock, 1975; Kay, 1979; Simpson *et al.*, 1983/84; Suttie *et al.*, 1984; Suttie & Simpson, 1985), and Scott *et al.* (2008a) hypothesised that the ensuing moderate energy intake imbalance between seasons mediates the observed variation in gestation length; a photoperiod-induced reduction in hind food intake during winter may impact on the ability of early-conceiving hinds to meet the increasing energy demands of the rapidly growing fetus during the last third of pregnancy.

However, although seasonal change of voluntary food intake (VFI) has been well documented for young growing red deer of both sexes and for adult stags (Pollock, 1975; Milne *et al.*, 1978; Kay, 1979; Simpson *et al.*, 1983/84; Suttie *et al.*, 1984; Suttie & Simpson, 1985; Suttie *et al.*, 1987; Loudon *et al.*, 1989; Semiadi *et al.*, 1994; Webster *et al.*, 2000), there is a paucity of such data for adult hinds (Blaxter *et al.*, 1974; Suttie & Simpson, 1985; Loudon *et al.*, 1989), and there appears to be no published data on seasonal VFI of pregnant red deer hinds.

1.2 Nature and scope of the investigation

This thesis reports on a study designed to investigate the effect of season and pregnancy status on VFI of adult red deer hinds throughout the gestating period. The study was undertaken as the first step in testing the hypothesis of Scott *et al.* (2008a) that early-conceiving hinds have a longer gestation length than those that conceive later because of seasonal variation in VFI, and hence fetal growth trajectory. The stated hypothesis is that pregnancy status does not affect the photoperiod-mediated reduction in VFI of red deer hinds during winter.

The literature review begins by emphasising that red deer evolved in temperate regions of Europe where seasonal variations in temperature and food availability are much greater than here in New Zealand; the few generations red deer have been in New Zealand are insufficient to overcome endogenous seasonal rhythms that are synchronised with the European environment. It then outlines known factors that affect calving date and VFI, before describing how hunger and satiety are regulated.

The body of the thesis describes how the study was managed, reports on the results from analyses of the data collected, and then discusses the results with reference to the findings from other studies. A concluding chapter summarises the main findings from the study and identifies its weaknesses and how it may have been improved, before suggesting areas of future research to substantiate inferences made. The concluding paragraph makes a recommendation to New Zealand deer farmers on the management of pregnant hinds.

Chapter 2: Review of the Literature

2.1 From Europe to New Zealand

Red deer (*Cervus elaphus*) evolved in temperate regions of Europe (Whitehead, 1972) where seasonal extremes in temperature and feed availability strongly influence animal survival. This has led to markedly seasonal annual rhythms of reproduction, growth, feed intake and pelage changes (Lincoln & Short, 1980; Suttie & Simpson, 1985). The first liberations of red deer in New Zealand occurred in the latter half of the 19th century and were primarily of the Scottish subspecies *C. e. scoticus* sourced from the UK. They thrived under predator-free conditions in large expanses of forest and alpine grasslands to rapidly establish wild populations throughout the country (Wodzicki, 1950). Through capture and enclosing behind fences these animals have formed the base of the New Zealand deer farming industry (Yerex, 1982).

In the northern latitudes of Europe the prevailing conditions have dictated a highly seasonal pattern of autumn conception and early summer calving for survival of the species (Lincoln & Guinness, 1973). Calves born very early or late in the season are less likely to survive as neonates than those born at the peak of calving (Iason & Guinness, 1985). Early born calves are subjected to a high risk of post-natal mortality due to inclement weather, and although feed availability is such that late-born calves are able to meet their nutritional needs for immediate survival, they have insufficient time to lay down the body reserves necessary to survive their first winter (Clutton-Brock *et al.*, 1982). However, under New Zealand lowland farming conditions, pasture quality and feed availability are generally high in spring and low in summer and autumn (Litherland *et al.*, 2002) resulting in a misalignment of peak pasture quality and the nutritional demands of a lactating hind and her offspring (see review: Asher *et al.*, 1996). To date, efforts to advance the calving date of farmed red deer appear to be partly offset by robust and complex adaptations of reproductive processes that have evolved to ensure offspring are born at the optimal time for survival. Few generations of red deer have been exposed to the New Zealand farming environment, and there has been insufficient time for selection pressure to have modified their inherent seasonality. The challenge for the New Zealand deer farmer, therefore, is to mitigate the effects of evolutionary adaptation of red deer to climatic

conditions in the northern latitudes of Europe so as to better align feed availability with nutritional demand of the lactating hind under New Zealand lowland pastoral conditions.

2.2 Factors affecting calving date

Time of conception has generally been regarded as the main driver of calving date, with gestation length of red deer considered to be genetically fixed at around 233 days (Guinness *et al.*, 1971; Kelly & Moore, 1977). Until recently, little attention has been paid to the possibility that time of parturition may be regulated by changes in gestation length (Asher, 2007). For instance, Guinness *et al.* (1978) reported that wild hinds had a 5-day longer gestation length than captive hinds and noted that this may have been due to captive animals receiving supplementary feeding. However, they concluded that gestation length varies little and that differences in calving date are most likely to be influenced by factors affecting the time of conception.

2.2.1 Conception date

Seasonal breeding is an adaptive response of animals to their environment to ensure that offspring are born at the optimal time for their survival. Red deer are ‘short day-breeders’, with onset of the breeding season occurring in autumn and parturition in summer (Lincoln & Short, 1980). In an effort to advance calving date a number of strategies to advance the period of conceptions have been applied to red deer hinds. Hormonal events regulating oestrous cycles of red deer are intrinsically the same as those of other domestic ruminant species and the procedures used are based primarily on those used for sheep (see review: Haresign, 1992).

Melatonin treatment

Photoperiod is the primary environmental cue that entrains the annual reproductive cycle of seasonally breeding animals. This was first noted for red deer by Marshall (1937) after the species was translocated from various countries in Europe to New Zealand. It was observed in all instances that when stags were translocated from one hemisphere to the other, they readily adjusted their cycles to those of the indigenous population. Similarly, hinds translocated from New Zealand to England adapted their

oestrous cycles to the English seasons within two years. Experimental evidence to corroborate that observation was subsequently reported from various studies that manipulated photoperiod in a number of temperate cervid species: red deer (Jaczewski, 1954; Suttie *et al.*, 1984; Suttie & Simpson, 1985; Webster & Barrell, 1985), sika deer (*Cervus nippon*: Goss, 1969a, b; Goss and Rosen, 1973) and fallow deer (*Dama dama*: Schnare & Fischer, 1987).

Melatonin is secreted from the pineal gland during the hours of darkness and is the principal transducer of photoperiodic information to the brain (see review: Arendt, 1986). When melatonin is administered during long photoperiods the recipient essentially perceives a short photoperiod. This has been demonstrated to induce early onset of the breeding season in a number of species: sheep (Kennaway *et al.*, 1982, Arendt *et al.*, 1983), goat (Chemineau *et al.*, 1986), white-tailed deer (*Odocoileus virginianus*: Bubenik, 1983), fallow deer (Asher *et al.*, 1988) and red deer (Adam & Atkinson, 1984; Webster & Barrell, 1985; Adam *et al.*, 1986; Fisher *et al.*, 1988). The methodology for delivering melatonin used in early studies was impractical in a commercial situation, as it involved the administration of daily doses of melatonin, either orally in the feed (Adam & Atkinson, 1984), or by injection (Webster & Barrell, 1985). The development of small constant-release implants (e.g. Regulin), developed for out-of-season breeding of sheep in Australia, provided a practical tool for deer. Treatment required only one or two implants in deer and advanced oestrus and conception by up to 8 weeks in fallow deer does (Asher *et al.*, 1988) and 4-5 weeks in red deer hinds (Fisher *et al.*, 1988; see review: Asher *et al.*, 1993). Although practical and effective, the use of melatonin implants is perceived as a high-intervention procedure and 'unnatural'. It has found little favour as a management tool to advance conception date in the New Zealand deer farming industry.

Gonadotrophin treatments

Whereas melatonin treatment alters the animal's perception of the prevailing photoperiod, administration of exogenous gonadotrophins (e.g. eCG, LH, FSH) or gonadotrophin-releasing hormone (GnRH) over-rides photoperiod control (by compensating for the lack of endogenous secretion from the pituitary gland during anoestrus) to stimulate ovarian activity before onset of the normal breeding season.

Exogenous progesterone (e.g. CIDR[®] device) plus equine chorionic gonadotrophin (eCG) in low doses (200 - 300 i.u.) has been used to effect 3-5 week advances in onset of oestrus in red deer (Adam *et al.*, 1985; Fisher *et al.*, 1986; Moore & Cowie, 1986; Fisher *et al.*, 1989) and fallow deer (Asher & Smith, 1987). However, the response was variable and, although ovulation was induced in a large proportion of hinds and does, fertility (as assessed from birthing records) was generally poor. Also, higher doses of eCG resulted in superovulatory responses (Fisher & Fennessy, 1987; Asher & Smith, 1987) and the inherent problems associated with the ensuing multiple births.

Low doses of GnRH administered over 48 h, either as pulsed injections or as continuous venous infusions, induced fertile oestrus in progestagen-primed anoestrous ewes, without the concomitant increase in ovulation rate associated with eCG (McLeod & Haresign, 1984). This approach has been tried in both red deer (Fisher *et al.*, 1986; Fisher *et al.*, 1989; Duckworth & Barrell, 1991) and fallow deer (Asher & Macmillan, 1986), but again, fertility was generally poor and Duckworth & Barrell (1991) attributed this to abnormal hormonal and behavioural patterns.

The use of progesterone priming in conjunction with low doses of eCG is commonly used to synchronise ovulation for artificial insemination and embryo transfer programmes and may be used to advance conception date when used in this context, but has found little favour in the New Zealand deer industry to advance conception date *per se*.

Early stag introduction

In the wild, red deer stags begin to rut about a month before hinds come into oestrus. The herding and roaring activity of the rutting stag is thought to promote a degree of oestrous synchrony within the hind harem (Lincoln & Guinness, 1973). However, in the farmed situation, stags are not usually joined with hinds until after calf weaning in March (Audigé *et al.*, 1999), thus denying hinds the early social interaction with stags that may happen in the wild.

Introduction of rams to anoestrous ewes (termed 'joining' in New Zealand) pre-conditioned by a period of isolation from rams induces, within a few minutes, an increase in pulse frequency of luteinising hormone (LH) secretion. This stimulates follicular growth and subsequently oestradiol secretion which, in turn, leads to an LH surge and ovulation (see review: Martin *et al.*, 1986). This phenomenon is referred to

as the ‘ram effect’ and is used widely in Australia as a management tool to induce lambing synchrony and for out-of-season breeding systems (Reeve & Chamley, 1984). Since the first published report of the ‘ram effect’ in sheep by Underwood *et al.* (1944), there have been numerous studies in a variety of species on the effect of joining males with anoestrous females: goats (Shelton, 1960; Ott *et al.*, 1980), pigs (Brooks & Cole, 1970), cattle (Skinner & Bonsma, 1964; Macmillan *et al.*, 1979; Scott & Montgomery, 1987), buffaloes (*Bubalus bubalis*: Gokuldas *et al.*, 2010), Eld’s deer (*Cervus eldi*: Hosack *et al.*, 1999), reindeer (*Rangifer tarandus*: Shipka *et al.*, 2002) and red deer (Moore & Cowie, 1986; Fisher *et al.*, 1995; Scott *et al.*, 2005).

Early stag joining is low intervention, low cost and ‘natural’, but the resultant advance in calving date in red deer is modest and variable. Moore & Cowie (1986) joined vasectomised stags with a group of adult hinds for 15 days before entire stags were joined on 22 March. They reported that more teased (20/39) than non-teased (5/42) hinds conceived within the first 10 days of joining. Likewise, Fisher *et al.* (1995) reported an 8 day advance in onset of ovarian activity in pubertal hinds that were joined with stags from 18 January compared to those remaining isolated from stags until after the commencement of the breeding season. In contrast, Scott *et al.* (2005) ran ‘spiker’ (15-18 month old) stags with pubertal hinds at a ratio of 1:8 from 15 January and reported no difference in mean conception date, or pregnancy rate, compared to pubertal hinds joined with the same ratio of ‘spiker’ stags on 2 March. However, young stags exhibit little rutting behaviour (Lincoln, 1971), and the authors considered that this may have influenced their ability to induce early oestrus in pubertal hinds. Also, there is evidence that the ‘ram effect’ is dependent on depth of anoestrous at time of male introduction (Martin *et al.*, 1980; Scott & Johnstone, 1994) and joining stags with pubertal hinds well in advance of their normal onset of oestrus may have nullified the ability of hinds to respond to stag introduction.

Early weaning

Lactating red deer hinds in the wild are reported to have reduced fertility (Guinness *et al.*, 1978) and later conception dates (Mitchell & Lincoln, 1973) than their non-lactating contemporaries, but this could be attributed to their poorer body condition at mating (Hamilton & Blaxter, 1980). Early weaning (i.e. before the rut) of farmed red deer in a drought year resulted in hinds conceiving earlier than those that were weaned post-rut (Pollard *et al.*, 2000). This was also attributed to the pre-rut weaned

hinds having a higher autumn body condition score (BCS) than those that were weaned post-rut. However, Loudon *et al.* (1983) suggested that the suckling stimulus of the calf has an inhibitory effect on resumption of ovarian activity in red deer, as has been shown in beef cows (Montgomery, 1982). This is corroborated for red deer from the data of Adam *et al.* (1985) who reported that lactating hinds started ovarian cycles 10 days later and conceived 16 days later than did weaned hinds, but considered that this was not likely to have been a body condition effect (Hamilton & Blaxter, 1980), because all hinds were in good body condition throughout the study. Also, Garcia *et al.* (2002) reported that progesterone levels remained basal for several months after calving, except in a hind that lost her calf just after parturition. That hind showed two consecutive oestrous cycles in the month following calving, and they suggested that this was indicative of the suckling stimulus having an inhibitory effect on the resumption of ovarian cyclic activity. Regardless of the causative mechanism, in all cases, hinds that were weaned pre-rut conceived earlier than contemporaries that were weaned post-rut. Early weaning is a non-invasive management practice that may be used to advance calving date on New Zealand deer farms.

Genetic selection

The red deer species has evolved within a wide range of latitudinal and longitudinal habitats, exposing them to large climatic variation and resulting in discrete populations with pronounced phenotypic and genotypic differences (i.e. subspecies). The red deer consists of at least 23 recognised subspecies which increase in size in a west-to-east cline from Western Europe to North America (Whitehead, 1993). Habitat and size variation between the subspecies may be associated with differences in onset of the breeding season and gestation length so as to synchronise calving with the time of greatest feed availability within each respective environment. For instance, Asher *et al.* (2000) reported that the mean onset of first oestrus was 9 days later for Western European red deer (*C. e. scoticus*) than for hybrids with North American wapiti (*C.e. nelsoni*). Furthermore, Scott *et al.* (2006) found that Western European red deer initiate rutting activity, and calve, 2 to 3 weeks later than Eastern European red deer (*C. e. hippelaphus*), indicating genetic differences between subspecies in response to the prevailing photoperiod.

There is also wide variation in conception date between individual deer of the same subspecies and the development of molecular DNA techniques such as single

nucleotide polymorphism (SNP) chips may allow screening of animals for genes that regulate seasonal reproduction (Nicoll, 2010).

2.2.2 Gestation length

Gestation length is defined as the period of time from conception to parturition and has generally been considered to be genetically fixed within limits for any given species. Although no one fundamental mechanism is considered to ensure maintenance of pregnancy or the initiation of parturition, it is thought that the initial mechanism for the timing of birth may be activated when certain prerequisite developmental events have occurred in the fetus (see review: Jenkin & Young, 2004). Thus, fetal genotype is probably the single most important determinant of gestation length (Racey, 1981). This is amply demonstrated in red deer subspecies where there is large variation in observed mating - birth intervals ranging from 233 ± 1 -4 days for Scottish red deer (Guinness *et al.*, 1971; Kelly & Moore, 1977) to 247 ± 5 days for North American wapiti (Haigh, 2001), with hinds gestating F1 crossbred fetuses having a mean gestation length intermediate between parental genotypes (Asher *et al.*, 2005b). Other modifiers of gestation length may include maternal age and body mass, fetal gender and environment (e.g. season, temperature and feed availability; Racey, 1981).

For example, Asher *et al.* (2005a) observed that differential nutrition during the last trimester of pregnancy influenced fetal development and that there was a negative correlation between duration of pregnancy and change in hind live weight between days 130 and 220 of pregnancy. Similarly, an increase in gestation length of 4-6 days was demonstrated in white-tailed deer by Verme (1965) when feed intake was severely restricted during pregnancy. Also, timing of births occurred earlier for Alaskan moose (*Alces alces*) cows with the thickest rump fat during pregnancy (Keech *et al.*, 2000). Asher *et al.* (2005a) hypothesised that a gestation length-compensatory mechanism ensured that parturition occurred when an optimal calf birth weight had been reached. However, no such mechanism was demonstrated when red deer hinds were artificially inseminated with semen from a wapiti bull and the authors considered that the higher growth requirements of a crossbred fetus may override any mechanism to control gestation length at the expense of calf growth rate (Asher *et al.*, 2005b). Mulley (1989) reported slower fetal growth rate and lower birth weight, but

no change in gestation length, when fallow deer were subject to modest levels of feed deprivation in the second and third trimesters of pregnancy, indicating that such compensatory mechanisms are not universal across cervid species. Furthermore, a number of studies have shown that nutritional deprivation during late pregnancy in sheep and cattle is invariably associated with a contradictory shortening of gestation length (Alexander, 1956; Alexander *et al.*, 1957; Bewg *et al.*, 1969; Tudor, 1972; Waldham *et al.*, 1979).

Time of mating has been reported to affect gestation length significantly in several ruminant species including sheep (Davies *et al.*, 1966), goats (Mellado *et al.*, 2000), cattle (Piedrafita *et al.*, 2000), dromedary (*Camelus dromedaries*: Elias *et al.*, 1991), alpacas (*Lama pacos*: Davis *et al.*, 1997), bison (*Bison bison*: Berger, 1992), reindeer (Rowell & Shipka, 2009), fallow deer (Asher *et al.*, 1988) and red deer (Garcia *et al.*, 2006; Scott *et al.*, 2008a), although the mechanism for this is yet to be elucidated. Asher *et al.* (1988) treated fallow deer with melatonin implants and achieved up to 8 weeks advancement in oestrus/conception which was associated with a significantly longer gestation period. They conjectured that the prevailing photoperiod may influence initiation of parturition. Berger (1992) proposed that late-breeding bison females in good condition shorten gestation to synchronise births with other females, but no similar adjustment was noted among females in poor condition. Garcia *et al.* (2006) observed that red deer hinds artificially induced to conceive early in the breeding season had longer gestation lengths than those conceiving later. They hypothesised that there was a compensatory mechanism in place that extended gestation period to ensure parturition matched food production. Similarly, Scott *et al.* (2008a) reported a negative correlation between conception date and gestation length such that for every 10 days difference in conception date there was a change in gestation length of from 1.9 – 4.9 days across different populations. Interestingly, they found no significant effect for hind age, hind live weight, calf gender, birth weight, sire genotype and year. They postulated that the observed effect of conception date on gestation length is mediated by the nutritional status of hinds entering the final trimester of pregnancy. That is, early conceiving hinds enter the final trimester of pregnancy when their VFI is lower than that of late conceiving hinds, resulting in differing fetal growth trajectories and thus different lengths of gestation.

2.2.3 Summary: calving date

A misalignment of quality feed availability and feed demand of the lactating hind and her growing calf is often seen as a limiting factor for efficient venison production under New Zealand pastoral farming systems. While there are tools available that may advance conception date by up to 2 months (e.g. melatonin implants), there is reluctance within the New Zealand deer farming industry to implement those that involve administration of exogenous hormones. It is perceived that such usage detracts from the New Zealand marketing image of being ‘clean and green’. Management practices that do not require the use of exogenous hormones are more modest in the gains they may achieve. Also, calving date is a function of both conception date and gestation length, and it has been found recently that for every 10 day advance in conception date, gestation length increases by about 3 days, exacerbating the situation. Little is known about how change in gestation length is achieved, but it is likely that there is an association with nutrition of the hind in late gestation.

2.3 Factors affecting voluntary food intake

As an adaptation to living in temperate zones with predictable seasonal cycles of food abundance in summer and scarcity in winter, many animals exhibit seasonal variation in VFI, body mass and energy metabolism that do not reflect actual changes in food availability, but are a function of physiological changes in response to predictors of the seasonal environment (see review: Loudon, 1994). Such an adaptation is thought to have evolved so that less energy is expended on foraging for food during times of scarcity (Kay & Staines, 1981). Seasonal animals are also assumed to maintain an appropriate body mass which varies depending on circumstances such as age, reproductive status or season (‘sliding set-point’). Body mass is thought to be maintained within this ‘set-point’ through changes in food intake or energy expenditure (see review: Keesey & Hirvonen, 1997). Evidence of such a mechanism was demonstrated in white-tailed deer (Ammann *et al.*, 1973) and red deer (Webster *et al.*, 2000) when dry matter intake (DMI) of diets containing a wide range of energy densities was altered by the animals so that energy intakes and growth rates were similar on all diets. Similarly, Scott *et al.* (2008b) reported that

DMI of lactating red deer hinds was significantly influenced by energy density of the diet, resulting in little difference in total energy intake between hinds fed pellets containing 10.3 or 12.5 MJME/kg DM. Also, in the study of Webster *et al.* (2000), energy intake of red deer changed with season, providing evidence that the ‘set-point’ changes with season. In a different approach, Ryg (1983) interpreted an inverse relationship between a hormonally-induced weight change and rate of weight change post-treatment as evidence for a functional ‘set-point’ for body weight in reindeer (*Rangifer tarandus tarandus*).

2.3.1 Endogenous circannual rhythms

Endogenous circannual rhythms act as internal clocks so that animals can adjust their physiology and behaviour to annual cycles of seasonal change in their environment. Photoperiod is the main source of predictive environmental information, or Zeitgeber, that entrains endogenous circannual rhythms that continue with a periodicity of about one year even in the absence of any external cues (see review: Gwinner, 1986). Compelling evidence of endogenous circannual rhythms was first demonstrated by Pongelley & Fisher (1957) in golden-mantled ground squirrels (*Spermophilus lateralis*) that were maintained for two years under constant photoperiod (light:dark, 12:12) and temperature conditions. Annual cycles of food intake, body condition and time of hibernation of these animals were similar to those of free-living squirrels. A seasonal endogenous rhythm has also been shown to regulate reproduction in sheep (Karsch *et al.*, 1989) and VFI in red deer (Simpson *et al.*, 1983/84; Brinklow & Loudon, 1990; Heydon *et al.*, 1993).

2.3.2 Photoperiod

The advantages of a mechanism whereby VFI matches that of food supply in animals living in highly seasonal environments are well recognised. Early studies by French *et al.* (1956) demonstrated a seasonal pattern of hunger in white-tailed deer such that VFI is maximal in summer and minimal in winter, and this cycle has since been reported for several ruminant species (see review: Rhind *et al.*, 2002). Initial evidence for photoperiodic entrainment of the seasonal change of VFI was provided by the translocation of deer from the northern to southern hemisphere (Marshall,

1937) and detailed studies on red deer subjected to artificial photoperiod have since confirmed this. Pollock (1975) imposed on a group of male red deer an artificial daily photoperiod sequence of normal amplitude but with a 6-month period; temperature was not controlled. Under this photoperiod regimen two cycles of increased VFI, growth and antler development were compressed into one year. VFI reached its peak as daily photoperiod approached its longest duration and declined after the shortest day. Further studies supporting photoperiodic entrainment of the seasonal pattern in VFI have been conducted on cattle, sheep and red deer (Peters *et al.*, 1978; Kay, 1979; Simpson *et al.*, 1983/84; Suttie *et al.*, 1984; Suttie & Simpson, 1985).

2.3.3 Temperature

Animals living in temperate habitats are subjected to yearly variation in photoperiod, temperature and availability of food. Although seasonal variation in VFI is entrained by photoperiod (Section 2.3.2) it may also be modified by temperature. Energy requirements and food intake increase with exposure to low temperatures and conversely decrease with exposure to heat (see review: Baile & Forbes, 1974) and thus regulate body temperature in accordance with the ‘thermostatic’ theory of Brobeck (1948).

Ullrey *et al.* (1969) conducted a study in which pregnant white-tailed deer does were kept in outdoor pens for 9 weeks during late winter/early spring and exposed to temperatures that varied between -32.4 and 10.4 °C. They found no significant relationship between daily maximum, minimum and mean temperatures and average daily VFI. However, in a second study there was a significant relationship between mean weekly temperature and weekly VFI (Ullrey *et al.*, 1970) in support of the ‘thermostatic’ theory.

In a study conducted in a less harsh environment where the mean daily winter minimum and maximum temperatures were 1.1 and 10.7 °C respectively, Webster *et al.* (1997) reported that there was no significant effect of housing on dry matter intake (DMI) and live weight gain (LWG) of red deer stags fed *ad libitum*, but animals kept outdoors on a restricted diet had a higher DMI and LWG than those kept indoors. They concluded that animals restricted to near zero growth need to eat more to counteract the effects of a colder environment. From relationships between the metabolisable energy intake (MEI) and LWG of red deer stags fed outdoors or

indoors during winter, Fennessy *et al.* (1981) calculated their maintenance energy requirements to be 0.85 and 0.57 MJME/kg LW^{0.75}/day, respectively.

2.3.4 Reproductive status

As discussed in the previous section (2.3.2), seasonal variation of VFI is mediated by photoperiod, independent of reproductive status. However, the lower amplitude of VFI cycles observed in castrated, compared to entire, red deer stags (Kay, 1979; Loudon *et al.*, 1989) indicates that gonadal steroids modulate amplitude of the VFI cycles. Anukulkitch *et al.* (2006) found also that gonadal status affects the pattern of VFI in Soay sheep. VFI of both entire and castrated rams was high under a 16 h light:8 h dark photoperiod (LP) and declined to a nadir between 12 and 18 weeks after shifting to a 8 h light:16 h dark photoperiod (SP). However, VFI of gonad-intact animals then recovered after 20 to 30 weeks exposure to SP during which time that of castrated animals remained low. This period coincided with testicular regression and declining blood testosterone concentrations, purportedly due to photorefractoriness of the gonad-intact rams, indicating that high levels of testosterone suppress VFI. Similarly, VFI of castrated sheep was depressed by intravenous infusions of oestradiol (Forbes, 1971).

Pregnancy is a dynamic state and to ensure reproductive success the energy demands of the developing fetus must be met at all stages of gestation. Nicol & Brookes (2007) calculated the total energy requirement for the entire pregnancy of a red deer hind to be 55 MJME/kg birth weight above maintenance. During the last third of pregnancy the fetal and maternal components of pregnancy gain about 70% of their final mass in reindeer (Roine *et al.*, 1982) and red deer (Adam *et al.*, 1988a), and it was estimated that the additional energy requirements for pregnant above non-pregnant hinds increases from 1.7 to 5.0 MJME/day during that time (Adam *et al.*, 1988b). Paradoxically however, VFI of cattle and sheep fed concentrates has been shown to decline in the last few weeks of pregnancy and this may be related to high circulating levels of oestrogens at this time (Forbes, 1971). There appears to be no published data on VFI of red deer hinds throughout gestation.

2.3.5 Physical limitation

Ruminants are distinct from other animals in that they have a four compartment stomach consisting of the rumen, reticulum, omasum and abomasum. Initially food is ingested without much chewing and then later the ingesta is regurgitated, masticated and re-swallowed in a repeated process known as rumination, with larger material being selectively regurgitated. This process not only breaks down the plant cell wall for easier fermentation in the rumen, but also reduces particle size for passage through the reticulo-omasal orifice (Van Soest, 1982). Thus, the amount of time spent ruminating is influenced by the type of diet consumed; high fibre, poor quality forage requires more rumination time than concentrates. Campling & Freer (1966) reported that physical limitations of gut capacity set an upper limit to food intake of cows, and hence the rate of ingesta passage through the reticulo-omasal orifice may limit voluntary intake of long roughages such as hay and dried grass. However, ruminal capacity is adaptable over a period of time and therefore unlikely to be a limiting factor to VFI over long periods (Baile & Forbes, 1974). Forbes (1969) reported a significant negative relationship between the volume of rumen contents and the volume of uterus plus other abdominal organs in sheep and considered that competition for abdominal space in obese and pregnant animals may limit VFI.

2.3.6 Summary: voluntary food intake

Many animals that inhabit temperate zones have seasonal cycles of VFI that continue in the absence of any external cues. This circannual endogenous rhythm is entrained by photoperiod and is an adaptation to living in an environment with predictable seasonal cycles of food availability. However, although photoperiod is the primary environmental factor regulating VFI, temperature may also affect hunger. Animals living in a cold environment require more energy to maintain body temperature than those living in a warm environment and thus eat more. Similarly, energy requirements to meet the demands of the growing fetus increase dramatically in the last third of gestation. However, little is known about the effect of photoperiod, hormones and physical limitations of the rumen on VFI of the pregnant hind. Does a pregnant hind eat enough to support the rapidly growing fetus, or is pregnancy maintained at the expense of her body reserves?

2.4 Regulation of hunger and satiety

To survive, all animals must continuously balance food intake with energy expenditure. Those living in temperate environments face the additional challenge of contending with seasonally fluctuating extremes of temperature and food availability. Although it has been known for some time that many animals living in such an environment have a ‘sliding set-point’ body mass which is maintained through changes in food intake or energy expenditure (section 2.3) and that VFI is mediated by photoperiod (section 2.3.2), precisely how food intake is regulated is yet to be fully elucidated.

2.4.1 Hypothalamus

Hetherington & Ranson (1940) made electrolytic lesions to the hypothalamus in rats and reported that “at least one form of pathological obesity is directly traceable to a primary lesion in the hypothalamus” confirming the ideas of many previous investigators. Microscopic examination of the lesions allowed them to observe that destruction of the ventral portion of the hypothalamus was the most effective site for affecting adiposity. From these initial observations the concept developed that the ventral portion of the hypothalamus (VMN) controlled satiety while the lateral area of the hypothalamus (LHA) controlled hunger. However, much of the research was carried out on rodent species (rats and mice) that do not have seasonal cycles of VFI and were subject to severe short-term food deprivation, or carried a genetic abnormality. Using hypothalamo-pituitary disconnected sheep, Lincoln *et al.* (2001) provided convincing evidence that arcuate hypothalamic systems were responsible for generating long-term rhythms of VFI, body weight and energy balance in a seasonal animal.

Discoveries on the role of the hypothalamus in control of hunger and satiety have been incremental in the last decade and it has been demonstrated that the concept of the LHA as the ‘hunger centre’ and the VMN as the ‘satiety centre’ is too simplistic. Many more hypothalamic nuclei and neuronal circuits that interact with the brainstem and higher cortical centres are involved in the regulation of hunger and satiety (see review: Suzuki *et al.*, 2010).

2.4.2 Leptin and ghrelin

Food intake of a healthy young rat is so precisely adjusted to its energy needs that its fat stores remain almost constant, but rats in which the hypothalamus has been destroyed eat ravenously and become obese. Brobeck (1946) suggested that food intake is controlled by a thermosensitive hypothalamic centre as part of the normal regulation of body temperature, but this was refuted by Kennedy (1953) who proposed that food intake is determined by some limiting factor involved in the synthesis or transport of fat, thus maintaining stability in body weight and fat stores: the 'lipostatic' theory.

It was not until four decades later that this concept was validated with the discovery of the hormone leptin (Zhang *et al.*, 1994) and subsequent research which found that the rate of leptin secretion is correlated with total fat mass of the animal (see review: Reidy & Weber, 2000). Leptin is expressed predominantly by white adipose tissue, but is expressed also in brown fat, muscle, mammary gland, stomach, pituitary, placenta, ovary and liver. It is thought that leptin from adipose tissue may act in the long-term, whereas stomach leptin acts rapidly and transiently. Plasma factors indicative of nutritional status such as glucose, amino acids and insulin that are released at the time of food intake stimulate leptin secretion (see review: Cammisotto *et al.*, 2010). Leptin acts in the arcuate nucleus (ARC) of the hypothalamus and inhibits expression of agouti-related peptide (AgRP) from neuropeptide Y (NPY) neurons and also activates pro-opiomelanocortin (POMC) cells to release melanocyte stimulating hormone (α -MSH) and promote satiety (Gao & Horvarth, 2007). Much of the research on leptin has been carried out on humans and laboratory rodents, but it is also well characterised in farm animals. Although the gene sequence varies between species, homology of the bovine leptin gene with that of human and mouse is 82% to 88% (see review: Wylie, 2010).

Ghrelin was initially purified from rat stomach cells and identified as an endogenous ligand specific to the growth hormone secretagogue receptor (Kojima *et al.*, 1999). However, it soon became evident that ghrelin played a significant role in the stimulation of feeding (Wren *et al.*, 2000) and is the only known peripherally active orexigenic peptide. Ghrelin is secreted by the stomach and stimulates NPY neurons in the ARC to silence POMC firing and express AgRP, which promotes hunger and feeding by reducing the anorectic effect of α -MSH (Gao & Horvarth,

2007). Synthesis and secretion of ghrelin are regulated by nutritional state; levels rise in anticipation of food and decrease postprandially. Exogenous administration of ghrelin either peripherally or centrally causes a rapid transient increase in VFI in rodents, humans and ruminants (see review: Roche *et al.*, 2008). Ghrelin is also considered to play a role in providing information on the long-term nutritional status of ruminants, since prolonged nutrient restriction in beef cattle results in a persistently elevated plasma ghrelin concentration (Wertz-Lutz *et al.*, 2008).

2.4.3 Anticipatory regulation

Animals that have evolved in temperate regions adjust their VFI in anticipation of the seasonal changes in food supply by responding to annual changes in photoperiod (section 2.3). These long-term (anticipatory) changes in energy regulation require pathways other than those known to mediate short-term homeostatic regulation. Experimental evidence from the Siberian hamster (*Phodopus sungorus*), a species that displays wide seasonal variations in fat accretion, VFI and reproduction, suggests that a seasonally appropriate body weight is continually reset according to photoperiod ('sliding set-point'; section 2.3) and that there is a photoperiod-induced change in leptin gene expression (see review: Adam & Mercer, 2001). More recently, Ebling & Barrett (2008) have hypothesised that thyroid hormone-dependent changes of hypothalamic connections and brain growth underlie seasonal cycles of food intake and body weight in the Siberian hamster. Furthermore, Bradley *et al.* (2010) have reported that changes in photoperiod alter the behavioural response to ghrelin and the ability of ghrelin to activate ARC NPY neurons in that species. In sheep, VFI is maximal during late summer/early autumn and is associated with increased expression of NPY (see review: Clarke, 2001). An *in vitro* study on sheep pineal explants by Zieba *et al.* (2011) has demonstrated that anorectic (leptin) and orexigenic (orexin B and ghrelin) peptides act on the pineal gland directly and its response to those hormones depends on daily photoperiod: during long days orexin B increased, and leptin decreased, melatonin secretion, while ghrelin had no effect; during short days leptin stimulated and ghrelin reduced melatonin secretion, while orexin had no effect; with the addition of leptin to ghrelin-treated explant cultures melatonin secretion was increased compared with cultures supplemented with ghrelin alone during both photoperiods. The authors proposed that in seasonally breeding animals leptin and

ghrelin, together, contribute to the integrative control of energy balance and reproduction.

2.4.4 Summary: hunger regulation

To modulate feeding, peripheral signals reflecting both the short- and long-term energy status of the animal must be transmitted to the hypothalamus.

It is known that two peptide hormones, leptin and ghrelin, play a major role in maintaining metabolic homeostasis in mammals. Their primary targets are the NPY neurons in the ARC of the hypothalamus; NPY is a potent orexigenic hormone. Ghrelin stimulates and leptin inhibits NPY expression and release and thus, the energy balance of animals is maintained partly by the antagonistic actions of these two hormones. Photoperiodic history is implicated in modulation of the effects of these hormones on neural pathways in seasonal animals.

2.5 Conclusion

Many animals that have evolved in temperate regions of the world respond to annual changes in daily photoperiod to adjust their VFI, body mass and energy metabolism in anticipation of the seasonal changes in temperature and food supply. When these animals are translocated to more hospitable climates their extant seasonal cycles may no longer reflect actual changes in temperature and food availability in their new environment, but are a function of an endogenous circannual rhythm involving complex physiological changes.

It is known that photoperiod is the primary source of predictive environmental information that entrains endogenous circannual rhythms, although they may be modified by other Zeitgebers such as temperature. Melatonin is secreted by the pineal gland during the hours of darkness and is the principal transducer of photoperiodic information to the brain, in particular, the hypothalamus. Within the hypothalamus lies the arcuate nucleus (ARC), which has been demonstrated to be responsible for generating long-term rhythms of VFI, body weight and energy balance in sheep. The hormones leptin and ghrelin provide information to ARC neurons of the energy status of the animal: ghrelin promotes hunger and leptin promotes satiety. Thus, the energy

balance of animals is maintained partly by the antagonistic actions of these two hormones.

To maximise the productivity of farmed venison supply systems requires that food availability matches energy demands at all times. For the hind and rapidly growing calf, energy demand is highest from late pregnancy through to weaning. The reproductive cycle of red deer has evolved to match perfectly food availability in the temperate regions of Europe. However, under New Zealand lowland farming systems pasture quality is often low during summer, limiting the genetic potential for calf growth. Better alignment of energy demand with food availability may be achieved by advancing calving date three or four weeks to coincide with the late-October spring flush of pasture growth.

Non-invasive management tools such as genetic selection for early-calving hinds and joining stags with hinds at early (late February) weaning have been adopted by many farmers resulting in earlier calving dates. However, advances in calving date have not always matched those expected from known conception dates. It seems that red deer have evolved a robust mechanism that ensures offspring are born at the optimal time for survival in the temperate zones of Europe: for every ten days advance in conception date, gestation length increases by about three days, and vice-versa. The mechanism for this adaptation is not understood, but it has been hypothesised that the observed effect of conception date on gestation length is mediated by the nutritional status of hinds entering the final trimester of pregnancy. It is known that there is a photoperiod-induced reduction of VFI in non-pregnant red deer hinds during winter. Therefore, energy intake of early-conceiving hinds may be insufficient to meet the requirements of a rapidly growing fetus during the last third of pregnancy, thus slowing fetal growth trajectory and delaying parturition.

The present study is the first step in testing that hypothesis. Do pregnant red deer hinds have a reduced VFI during winter?

Chapter 3: Materials and Methods

3.1 Experimental design overview

The study was conducted in a single year at the AgResearch Invermay Research Centre located in Mosgiel, New Zealand (latitude 45° 51' S) and involved individual housing of pregnant and non-pregnant adult (> 3 year-old) red deer hinds from April to November, during which time they were fed an *ad libitum* diet of deer pellets plus 5% lucerne chaff. Food intake was monitored daily to assess the effect of pregnancy status and season on voluntary food intake.

All animal manipulations were approved by the AgResearch Invermay Animal Ethics Committee (Project Number 11700), as required in New Zealand by the Animal Welfare Act 1999. All procedures were conducted by fully trained staff from the Invermay Agricultural Centre and in accredited facilities (NZQA accreditation scheme).

3.2 Animals and management

After weaning in early March 2009, 12 F1 *Cervus elaphus hippelaphus* (E) x *C. e. scoticus* (W) red deer hinds were transported from the Invermay Hill Deer Farm to the Invermay Flat Deer Farm. They were run with 8 F1 (E x W) hinds already resident there that had not reared a calf and had been used previously for intensive blood sampling experiments, so were well habituated to handling. All twenty hinds were grazed on short pasture and gradually, over a 2-week period, habituated to eating standard deer pellets. The initial offer of pellets was 100 g per hind per day and increased daily in increments of 100 g per hind per day to a maximum of 1.5 kg pellets per hind per day.

Care was taken to ensure allocation of hinds to treatment groups was balanced for hind history and live weight (LW). Eight hinds were allocated to remain non-pregnant (NP) and be housed indoors from 25 March (once habituated to pellets) until the end of the study. It was considered that stress associated with becoming accustomed to indoor housing may perturb the synchronised ovulation necessary for fixed-time artificial insemination; therefore, the remaining twelve hinds (P) remained outside on

short pasture and were fed pellets until 7 days post-artificial insemination. From the pool of twelve P hinds eight were selected, on their perceived suitability for indoor housing, to remain indoors from 8 April until about 24 h post-calving. The remaining four P hinds were kept as 'reserves' and were fed pellets at pasture until it was evident they would not be needed for the study. They then went back under normal farm management. Pregnancy status of P hinds was determined by rectal ultrasound scanning on 1 May and 23 June.

Hinds calved indoors to enable accurate calculation of gestation length; hinds and calves were weighed within 24 h of parturition before hind-calf pairs were returned to pasture. NP hinds remained indoors until the last P hind had calved.

3.3 Oestrous synchronisation and artificial insemination

Twelve P hinds received a 12-day hormone treatment to synchronise ovulation for fixed-time artificial insemination. On 18 March (Day 0), hinds received an intravaginal progesterone-releasing device (Eazi-breed CIDR[®] type G; Pfizer New Zealand Ltd., Mt Eden, Auckland, NZ) which was replaced by a second CIDR[®] device on Day 9. The second CIDR[®] device was removed between 1000 and 1030 h on Day 12 and the hinds concurrently injected with 180 i.u. equine chorionic gonadotrophin (Folligon, Intervet, Lane Cove, NSW, Australia).

Transcervical artificial insemination began at 1800 h on 1 April, 56 h after CIDR[®] device removal, using cryopreserved semen from a single E stag. Pregnancy status was ascertained by rectal ultrasonography (5 MHz linear array transducer; Aloka SSD210: Aloka Co. Ltd., Japan) 28 and 83 days later.

3.4 Indoor pens

Sixteen indoor pens were located in a single covered, ventilated building that was adjacent to outdoor exercise yards and had raceway access to a weigh-box and deer handling facility (pneumatic crush). Pens (approximately 6 m²; Figure 3.1) had a concrete floor covered in deep-litter sawdust and were constructed with panel walls so that visual contact could be maintained between neighbouring hinds in adjacent pens. Natural lighting, provided by skylights, was supplemented by artificial lights that were timed to automatically switch on at sunrise and off at sunset each day. Data

published by the Royal Astronomical Society of New Zealand (<http://www.rasnz.org.nz/>) were used to set sunrise and sunset times for the lights, with no allowance made for Civil Twilight.

Each pen was provided with a wooden food bin and a water nose-trough fitted with a float valve such that water was available *ad libitum*; both were fixed to a wall at a height of approximately 1 m (Figure 3.1). Faeces were removed and the sawdust raked daily; all sawdust in each pen was replaced at least once per month to prevent build-up of ammonia fumes from urine.

3.5 Feeding

Throughout the period of indoor confinement the diet consisted of a commercial pelleted deer food (Reliance Deer Nuts, Combined Rural Traders, Yaldhurst, Christchurch, NZ; Tables 2.1 & 2.2) plus 5% by weight lucerne chaff (10.5 MJME/kg DM; 22.9% crude protein) for adequate roughage to ensure maintenance of rumen function. Food not eaten (refusal) was collected each day and new food offered while the hinds were in outside exercise yards. To avoid acidosis from grain overload hinds were initially offered 1 kg pellets plus 5% lucerne chaff. Thereafter the food ration was adjusted to appetite daily according to the rule: if the refusal was < 10% of food offered, the new ration was increased by 200 g; if the refusal was > 10% of food offered, the ration remained the same as that on the previous day. Once per week a sample of the residual food was collected and weighed before and after drying for 24 h at 65° C to remove water and ascertain dry matter (DM) percentage; the value obtained was used to calculate daily DM intake of the hinds for that week.

Figure 3.1: Indoor pen (approximately 6 m²) with deep-litter sawdust, food bin and water nose-trough.



Table 3.1 Ingredients of the commercial pelletised ration expressed as a percentage of the total weight as fed.

Ingredient	% total weight (as fed)
Barley	69.7
Wheat	17.0
Soybean meal	9.0
Limestone (36% Ca)	2.5
Molasses	1.2
Salt	0.6

Table 3.2 Dry matter (% DM), total energy (MJME/kg DM) and nutrient composition of batches of the pelletised ration expressed as a percentage of dry matter (% DM).

Nutrient	As tested by manufacturer	Batch #1 6/3-15/4	Batch #2 16/4-5/5	Batch #3 6/5-21/6	Batch #4 22/6-24-9
Dry matter (%)	88.7	88.7	87.3	88.1	87.8
Total energy (MJME/kg DM)	12.7	12.3	13.0	12.7	13.0
Lipid	1.8	1.7	2.5	5.3	5.6
Crude protein	15.0	16.3	12.8	13.9	13.6
Starch	51.4	46.8	53.3	51.1	55.3
Acid detergent fibre	5.1	4.6	5.9	6	4.1
Neutral detergent fibre	18.4	20.8	22.5	19.3	20.3
Ash	5.7	2.2	1.8	1.7	2.0

3.6 Weighing and blood samples

Hinds were weighed to the nearest 0.5 kg and assessed for body condition score (BCS) every fortnight. BCS was based on a 5-point scale (1 = emaciation and 5 = obesity) as described by Audigé *et al.* (1998) and was assessed by visual and palpation appraisal of the spine, sacrum and wings of the pelvis.

From 23 April until 5 November, blood samples were collected at four-week intervals via jugular venepuncture into 10 ml evacuated tubes containing K₃ EDTA as an anticoagulant. Because of the purported pulsatile nature of ghrelin and leptin secretion, hinds were bled at 20 minute intervals over one hour, beginning at 1330 h on each collection date. The samples were kept on ice until centrifuged at 4 °C for 15 min at 2,000 g within two hours of collection. Plasma was pipetted into separate 1 ml aliquots for measurement of leptin and active ghrelin concentrations. In addition, plasma aliquots for ghrelin analysis were acidified with 50 µl of 1 N HCl and 10 µl of phenylmethylsulfonyl fluoride was added as a protease inhibitor to preserve the integrity of the octanoyl moiety of ghrelin, as required to measure the concentration of active ghrelin. Plasma was stored at -20 °C until assayed.

3.7 Hormone assays

Twenty-minute plasma samples were pooled for each animal on each sampling date before assay. Samples were thawed and mixed by a vortex stirrer before 250 µl of the 0, 20, 40 and 60 minute aliquots was pipetted into a separate tube to make pooled-hour plasma aliquots. Plasma concentrations of leptin and ghrelin were then measured from the pooled-hour plasma aliquots in duplicate 100 µl samples using commercially available radioimmunoassay (RIA) kits. All procedures were carried out in accordance with the manufacturer's protocol. The precipitate was collected by centrifugation at 4 °C for 25 minutes at 2,500 g and the supernatant discarded. Assay tubes containing pellets were counted for 1 minute on an automatic gamma counter (Wallac Wizard 1470, Perkin Elmer, Wellesley, MA, USA).

Plasma leptin concentrations were measured using a multi-species leptin RIA kit (LINCO Research, Cat. # XL-85K, St. Charles, MO, USA). This kit has been validated previously for cervids (sika deer: Suzuki *et al.*, 2004; reindeer: Soppela *et al.*, 2008; red deer: Gaspar-López *et al.*, 2009). The antibody used in the kit was

raised against human leptin in guinea pigs and the protocol recommends that ng/ml human equivalent (HE) is used as the unit of measure. The limit of sensitivity for the multi-species leptin kit is 1.0 ng/ml HE. Intra- and inter-assay coefficients of variation were 4.8 and 2.7% respectively.

A rat active ghrelin RIA kit (LINCO Research, Cat. # GHRA-88HK, St. Charles, MO, USA) was used to measure plasma active ghrelin concentrations. The kit utilises an antibody which is specific for the biologically active form of ghrelin with the octanoyl group on serine 3 and has a sensitivity of 7.8 pg/ml. Prior to analysing experimental samples, the kit was validated for cervine plasma by demonstrating parallelism to the standard curve of serially diluted cervine plasma (Figure 3.2). The intra- and inter-assay coefficients of variation were 11.2 and 7.8% respectively.

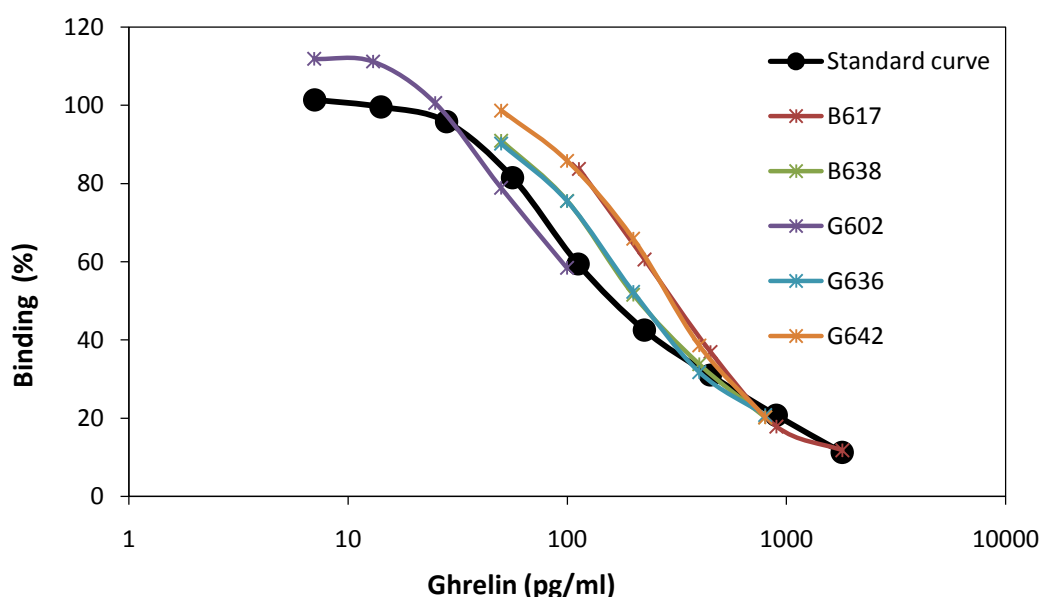


Figure 3.2 Parallelism between percentage binding for serial dilutions of cervine plasma from 5 hinds and the standard curve generated from the rat active ghrelin RIA kit.

3.8 Statistical analyses

Data from before 27 April, while hinds were building up to an *ad libitum* food intake, were not included in any of the analyses.

Effect of pregnancy status on changes in mean live weight (LW), body condition score (BCS) and VFI during specific time periods, and on plasma hormone concentrations at each sampling date, were analysed by analysis of variance (ANOVA), separately, fitting a term for pregnancy status. The time frames for VFI analyses were synchronised about date of parturition to compensate for the large variation in parturition date. For NP hinds, Day 0 was taken as the mean parturition date of P hinds.

When calculating change in VFI between seasons, mean VFI over 3 days (except for Days -5 and 0 when the VFI recorded only on those days was used) around the start and end date of the specified times was used to allow for large daily variation of individual hind intake. For example, VFI for Day -200 was calculated as the average VFI value of Days -201, -200 and -199. A semi-parametric linear mixed model with smoothing spline was applied to the mean VFI data using REML in the statistical package GenStat Version 11. Pregnancy status (Trtmnt), day of year (DOY) and the interaction term (Trtmnt.DOY) were fitted as fixed effects. Individual hind (ID) and the interaction ID.DOY were fitted as random model terms. The covariance structure was defined by ID and ID.DOY by allowing unrestricted correlation structure, and the structure formed by definition of the whole matrix. The initial values for covariance matrix terms were determined by estimates from running the same model but with no, or simple, covariance structure. An overall spline (termed DOY in the spline model), separate treatment splines (Trtmnt.DOY) and individual hind splines (ID.DOY) were also fitted as part of REML.

Webster *et al.* (2000) developed equations to model the way metabolisable energy intake for an animal at time t ($MEI_{(t)}$) is partitioned into energy for maintenance of metabolic live weight (E_m) and energy required for growth over the next day (E_g). If E_m ($MJ/kg^{0.75}$) is the amount of energy required to maintain 1 kg of metabolic body weight ($LW^{0.75}$), E_g (MJ/kg LWG) is the energy required to increase body weight (W) by 1 kg and E_r (MJ) is net energy retained in a growing animal, then:

$$1) \quad \text{MEI}_{(t)} = E_m \times \text{LW}^{0.75}_{(t)} + E_{r(t)}$$

$$2) \quad W_{(t+1)} = W_{(t)} + E_{r(t)} / E_g$$

We can eliminate $E_{r(t)}$ between these equations and denote change in live weight by $\text{LWG}_{(t+1)}$, giving

$$\begin{aligned} 3) \quad \text{LWG}_{(t+1)} &= W_{(t+1)} - W_{(t)} \\ &= (\text{MEI}_{(t)} - E_m \times \text{LW}^{0.75}_{(t)}) / E_g \end{aligned}$$

Equation 3) was fitted by multiple linear regression of $\text{LWG}_{(t+1)}$ on $\text{MEI}_{(t)}$ and $\text{LW}^{0.75}_{(t)}$ for set time periods denoting autumn, winter and spring to P and NP hinds separately. E_m and E_g were then calculated from estimates of the regression models and using equation 3) thus:

$$\begin{aligned} \text{LWG}_{(t+1)} &= (1 / E_g) \times \text{MEI}_{(t)} - (E_m / E_g) \times \text{LW}^{0.75}_{(t)} \\ &= \beta_2 \times \text{KJME} + \beta_1 \times \text{LW}^{0.75}_{(t)} \end{aligned}$$

$$E_m = \beta_1 / \beta_2 \times (-1)$$

Separate log likelihood ratio tests comparing regression models were then used to test the effect of pregnancy status, and season, on the maintenance requirement of hinds.

Regression lines were fitted to the mean data of each hind over the entire study period to explore the relationships between plasma hormone concentrations, gestation length and the variables reported.

Chapter 4: Results

4.1 General

One of the NP hinds did not adapt to being confined indoors and was removed from the study. The remaining hinds appeared to become well habituated to indoor housing conditions and took about two weeks to stabilise their *ad libitum* intake. One of the P hinds lost her pregnancy somewhere between the first (1 May) and second (23 June) ultrasound scan and her data were not included in the analysis. The remaining seven P hinds all had an unassisted calving and produced healthy singleton calves with birth weight ranging from 7.0 – 10.5 kg (mean = 9.8 kg), which is within the range expected from hinds grazed at pasture.

4.2 Live weight and body condition score

Mean live weight (\pm SEM) of P and NP hinds on 27 April (Day 117) was 117.1 ± 6.0 kg and 124.1 ± 10.3 kg respectively. A number of NP hinds went through large fluctuations of VFI and lost weight during an initial ‘settling in’ period, before regaining that weight; this was not so apparent in P hinds (Figure 1). This resulted in a significant difference of 103 g/day (SED 40 g/day; $P < 0.05$) between P and NP hinds in mean live weight gain (LWG) during the first 42 days of the study (autumn). On average, P hinds gained 75 g/day while NP hinds lost 27 g/day between late April and early June (Days 117 – 159). Mean live weight (\pm SEM) of both treatment groups then increased through to the last weighing day before the start of calving (9 November, Day 313), being 146.8 ± 9.9 kg and 147.9 ± 13.9 kg for P and NP hinds respectively. There were no significant differences between groups in rate of mean live weight change over specific time periods during winter and spring (Table 4.1).

Table 4.1 Mean hind live weight changes (g/day \pm SED) over specified time periods of the study where Day 1 = 1 Jan. (N.S., not significant; *, $P < 0.05$).

Time period (1 = 1 Jan)	Pregnant	Non-pregnant	SED	P
117 - 159 (Autumn)	75	-27	40	*
159 - 243 (Winter)	108	123	36	N.S.
243 - 313 (Spring)	230	207	61	N.S.

Table 4.2 Mean hind body condition score changes (BCS units/day \pm SED) over specified time periods of the study where Day 1 = 1 Jan. (N.S., not significant; *, $P < 0.05$)

Time period (1 = 1 Jan)	Pregnant	Non-pregnant	SED	P
117 - 159 (Autumn)	0.014	0.005	0.004	N.S.
159 - 243 (Winter)	0.002	0.002	0.001	N.S.
243 - 313 (Spring)	-0.001	0.006	0.002	*

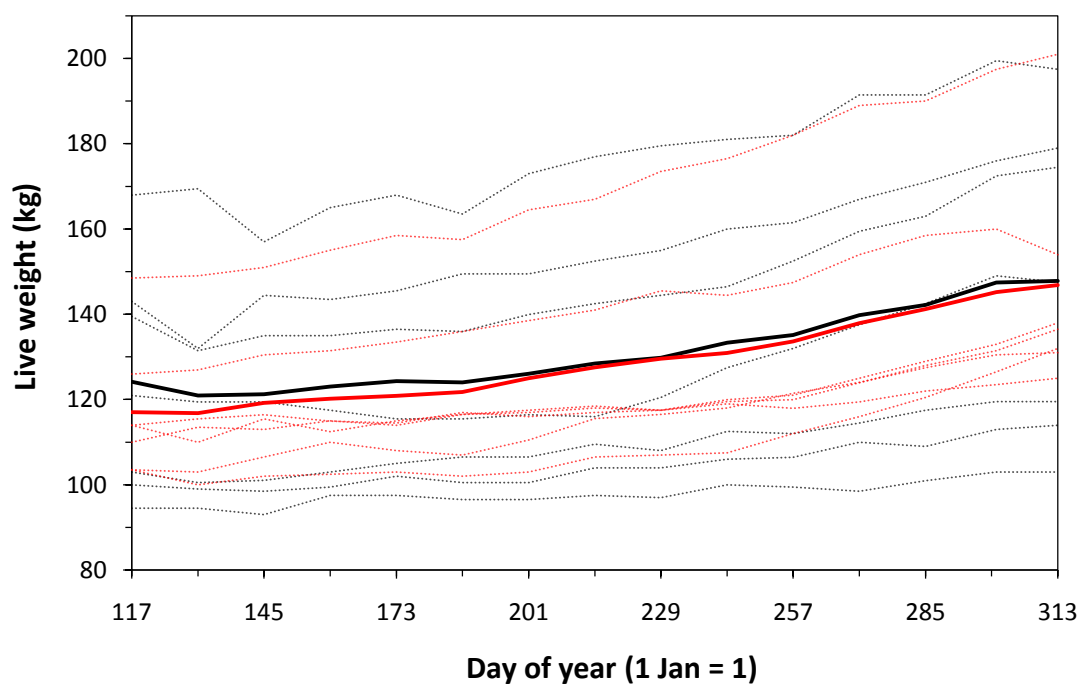


Figure 4.1 Profiles of individual (dotted line) and mean (solid line) live weight (kg) of pregnant (red) and non-pregnant (black) hinds.

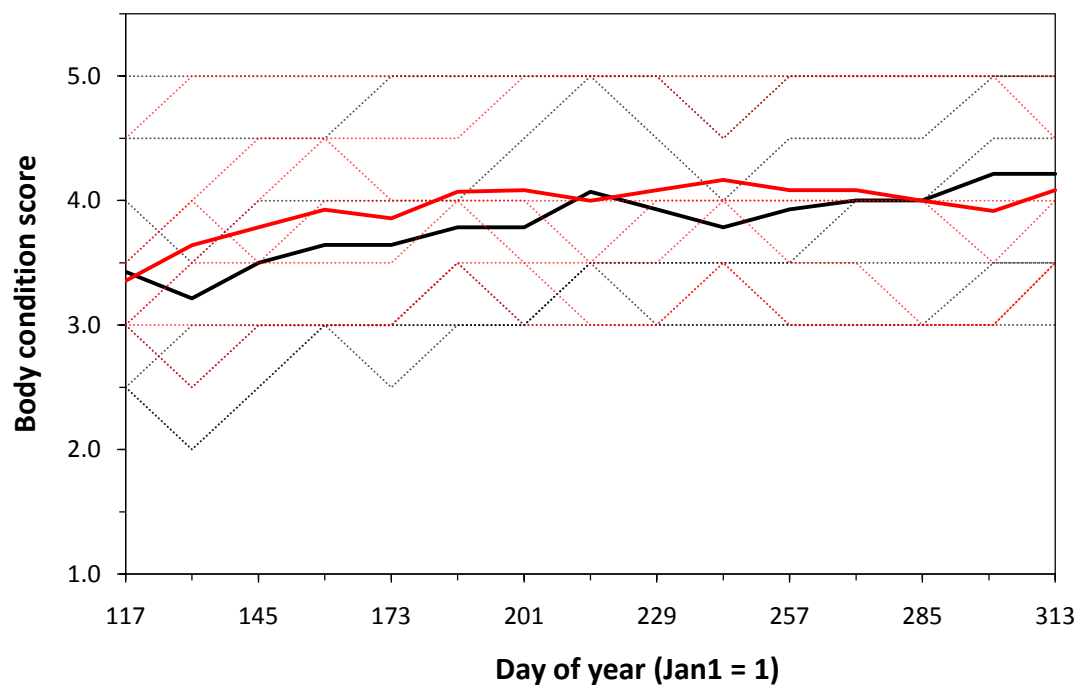


Figure 4.2 Profiles of individual (dotted line) and mean (solid line) body condition score (1 = emaciated, 5 = obese) of pregnant (red) and non-pregnant (black) hinds.

Mean body condition score (BCS) of hinds in both groups was higher on 9 November (P, 4.0 ± 0.6 ; NP, 4.2 ± 0.9) than on 27 May (P, 3.4 ± 0.6 ; NP, 3.4 ± 1.1), but the pattern of BCS change during the study differed with pregnancy status (Figure 4.2). Both P and NP hinds gained body condition during autumn and winter ($P > 0.05$), but mean BCS of P hinds decreased, whereas that of NP hinds increased, during spring ($P < 0.05$; Table 4.2).

4.3 Voluntary food intake

Pregnancy status of the hinds had no significant ($P > 0.05$) effect on mean voluntary food intake (VFI) throughout the study except for the last five days before parturition when VFI of P hinds decreased dramatically ($P < 0.001$). This relationship held when VFI was expressed as both absolute intake (MJME), or when adjusted for metabolic live weight (MJME/kg LW^{0.75}, Table 4.3). It is noted however, that there was a trend for P hinds to have lower VFI values for the entire duration of the study (Figure 4.3). Hind history (habituated or not habituated to handling) did not have a significant effect on hind VFI ($P > 0.05$) when included in the model.

There was considerable between-hind and between-day variation in VFI. For example, one hind rarely exceeded a daily dry matter intake (DMI) of 1.6 kg while another regularly ingested more than 3.5 kg. Between-day intake of individual hinds often varied by more than 0.5 kg DM, with hinds on higher intakes, in particular, going through 'feast and famine' cycles (Figure 4.4).

Mean (\pm SEM) VFI of hinds over three consecutive days in early-autumn (30 April – 2 May), mid-winter (30 June – 2 July) and late-spring (31 October – 2 November) were 0.724 ± 0.054 MJME/kg LW^{0.75}, 0.578 ± 0.029 MJME/kg LW^{0.75} and 0.686 ± 0.034 MJME/kg LW^{0.75} respectively. On average, hind intake decreased by 0.146 ± 0.060 MJME/kg LW^{0.75} from autumn to winter ($P < 0.05$) and increased by 0.107 ± 0.035 MJME/kg LW^{0.75} from winter to spring ($P < 0.01$), seemingly aligned with the seasonal change in day length (Figure 4.5).

Table 4.3 Mean (\pm SED) change in hind daily voluntary food intake (VFI) over specified time periods of the study as calculated from regression analysis of the predicted mean daily voluntary food intake (VFI) and expressed as a) MJME and b) MJME/kg LW^{0.75} where Day 0 = day of parturition. Data have been normalised around days from calving to compensate for the wide variation in calving dates. Mean parturition date of the pregnant hinds (20 Nov) was taken as Day 0 for non-pregnant hinds. (N.S., not significant; ***, $P < 0.001$)

a) Mean change in daily VFI (MJME) over specified time periods of the study

Days before parturition	Pregnant	Non-pregnant	SED	P
200-150	-6.507	-9.597	3.541	N.S.
150 -100	0.993	2.301	2.607	N.S.
100-50	2.746	5.880	2.136	N.S.
50-20	3.212	0.939	3.430	N.S.
20-5	-3.095	-0.906	5.370	N.S.
5-0	-10.347	6.419	3.956	***

b) Mean change in daily VFI (MJME/kg LW^{0.75}) over specified time periods of the study

Days before parturition	Pregnant	Non-pregnant	SED	P
200-150	-0.202	-0.251	0.103	N.S.
150 -100	0.001	0.042	0.067	N.S.
100-50	0.046	0.107	0.054	N.S.
50-20	0.066	-0.001	0.084	N.S.
20-5	-0.073	-0.040	0.123	N.S.
5-0	-0.241	0.170	0.094	***

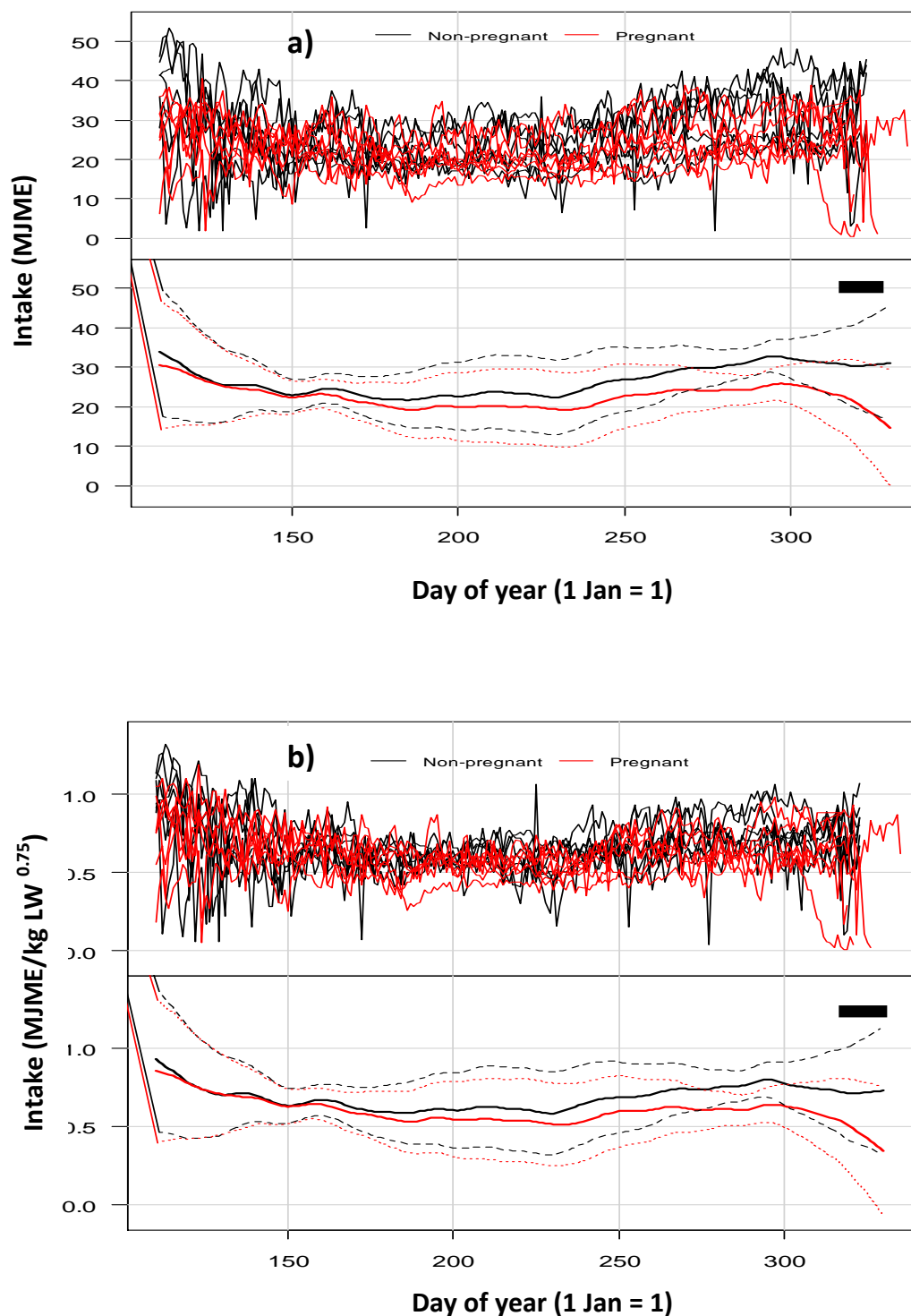


Figure 4.3 Profiles of daily individual voluntary food intake (VFI) (top panel) and predicted mean VFI (bottom panel) of pregnant (red) and non-pregnant (black) hinds expressed as a) MJME and b) MJME/kg LW^{0.75} where Day 1 = 1 January. Dotted lines represent SEM from the spline model. The solid bar represents the period of parturition.

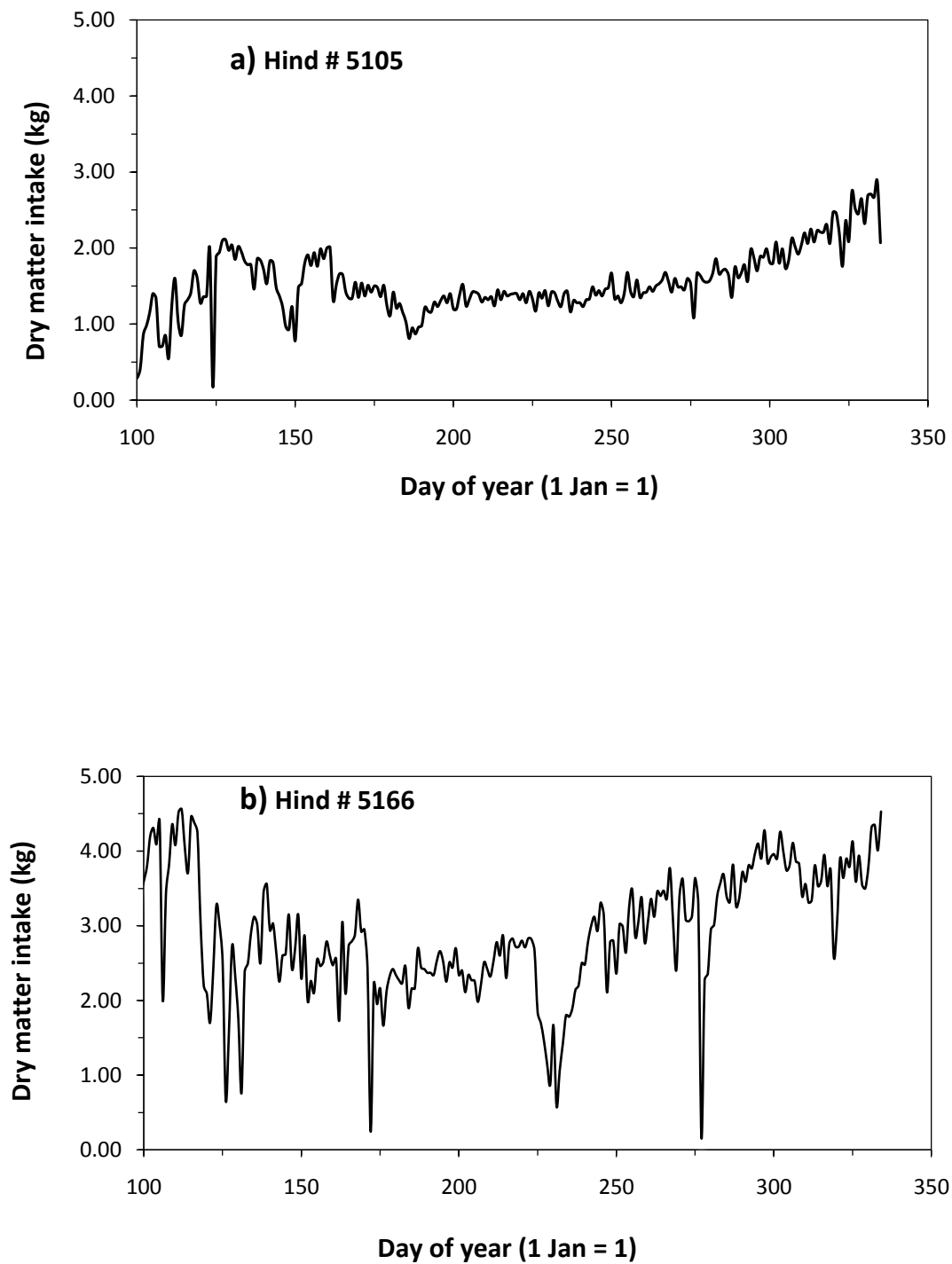


Figure 4.4 Examples of daily dry matter intake (kg) from two individual hinds demonstrating a) low mean intake with little daily variation and b) high mean intake with large daily variation.

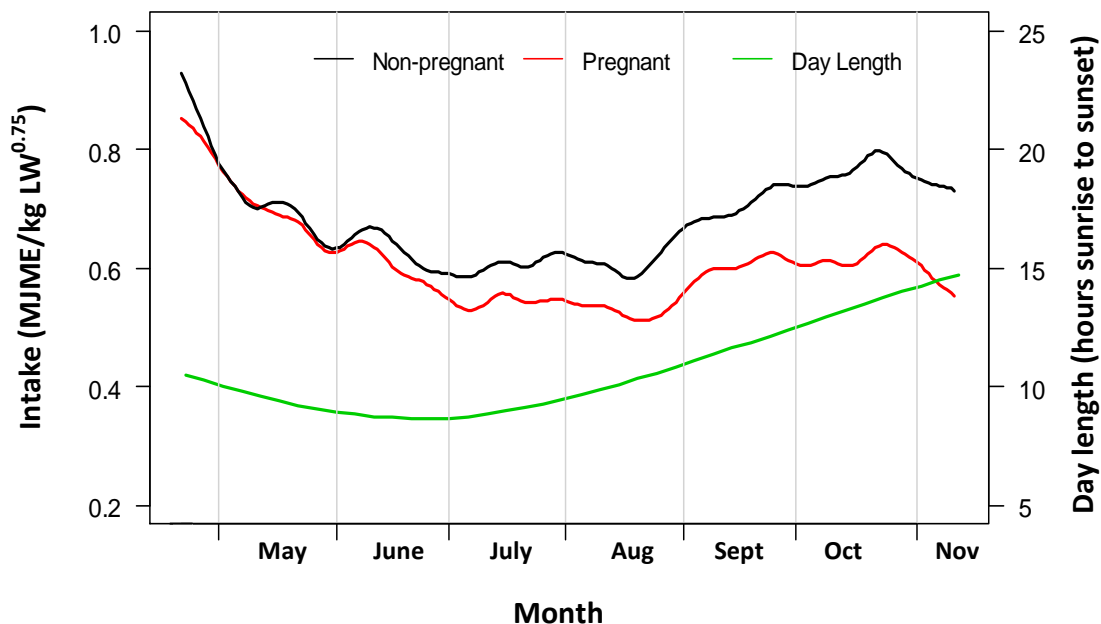


Figure 4.5 Predicted mean daily voluntary food intake (MJME/kg LW^{0.75}) of pregnant and non-pregnant hinds relative to day length (hours between sunrise and sunset) during indoor feeding.

The mean amount of energy required to maintain 1 kg of metabolic body weight (E_m) and to increase live weight by 1 kg (E_g) was calculated from estimates of regression models regressing daily live weight gain on metabolic live weight (LW^{0.75}) and metabolisable energy intake (MEI) as outlined in Section 3.8. The estimates were obtained by fitting the data of P and NP hinds for set time periods corresponding to autumn, winter and spring to the same model, separately (Table 4.4). The estimates of E_m for NP hinds in autumn may be artificially high because two hinds in that group were gluttonous at the beginning of indoor confinement, had widely fluctuating intakes and lost body weight. It is worth noting that standard error of the mean (SEM) values are high for both groups in winter and autumn, but not spring. Separate likelihood ratio tests were then used to test the effect of pregnancy status, and season, on E_m of hinds. A significant amount of the extra variation was explained by pregnancy status in winter and spring ($P < 0.01$), but not autumn ($P > 0.05$). Overall, there was a significant difference ($P < 0.0001$) in the regression relationships between seasons for the maintenance requirements of hinds.

Table 4.4 Multiple linear regression of daily live weight gain regressed on metabolic live weight (at the start of each set time period) and mean metabolisable energy intake over set time periods corresponding to autumn, winter and spring. E_m (\pm SEM) is the mean amount of energy required to maintain 1 kg of metabolic body weight ($\text{MJ/kg}^{0.75}$) and E_g (\pm SEM) is the mean amount of energy required to increase live weight by 1 kg (MJ/kg). Separate log likelihood ratio tests comparing regression models were then used to test the effect of pregnancy status on the maintenance requirement of hinds at each season with P being the probability that treatment does not explain the variation between fitted lines. (N.S., not significant; **, $P < 0.01$)

Time period (1 = 1 Jan)	Pregnant				Non-pregnant				P
	E_m ($\text{MJ/kg}^{0.75}$)	SEM	E_g (MJ/kg)	SEM	E_m ($\text{MJ/kg}^{0.75}$)	SEM	E_g (MJ/kg)	SEM	
(Autumn)									
117-159	0.377	0.369	138.2	160.2	0.818	0.242	148.9	259.2	N.S.
(Winter)									
159-243	0.292	0.251	89.99	78.22	0.343	0.279	78.98	79.85	**
(Spring)									
243-313	0.410	0.062	40.98	9.75	0.487	0.068	45.71	11.56	**

4.4 Leptin

Pregnancy status had no significant effect on mean plasma leptin concentration at any of the sampling times (Table 4.5) and there was no discernable seasonal pattern of circulating leptin concentration (Figure 4.6).

Intake of individual hinds was not associated with their plasma leptin concentration (Figure 4.7). However, there was a significant relationship between BCS and circulating leptin concentration ($r^2 = 0.411$, $P < 0.01$, Figure 4.8), such that, for every 0.5 unit increase in mean BCS mean circulating leptin concentration increased by 0.275 ng/ml human equivalents (HE).

Table 4.5 Mean concentration of plasma leptin (ng/ml HE \pm SED) at each sampling date. (N.S., not significant)

Sampling date	Pregnant	Non-pregnant	SED	P
23 Apr	1.881	2.310	0.335	N.S.
21 May	1.899	2.093	0.274	N.S.
17 Jun	1.940	1.844	0.224	N.S.
16 Jul	2.074	2.350	0.491	N.S.
13 Aug	2.107	2.123	0.372	N.S.
10 Sep	2.326	2.271	0.383	N.S.
8 Oct	2.090	2.263	0.385	N.S.
5 Nov	1.949	2.203	0.390	N.S.

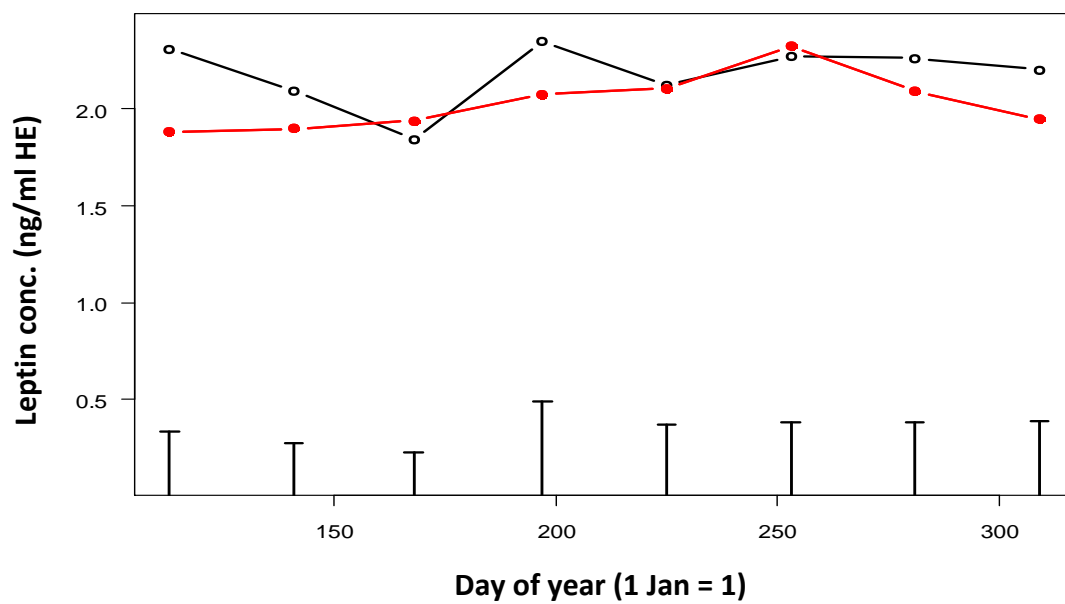


Figure 4.6 Mean plasma leptin concentration (ng/ml HE) of pregnant (red) and non-pregnant (black) hinds. The error bars are the SED at each sampling date.

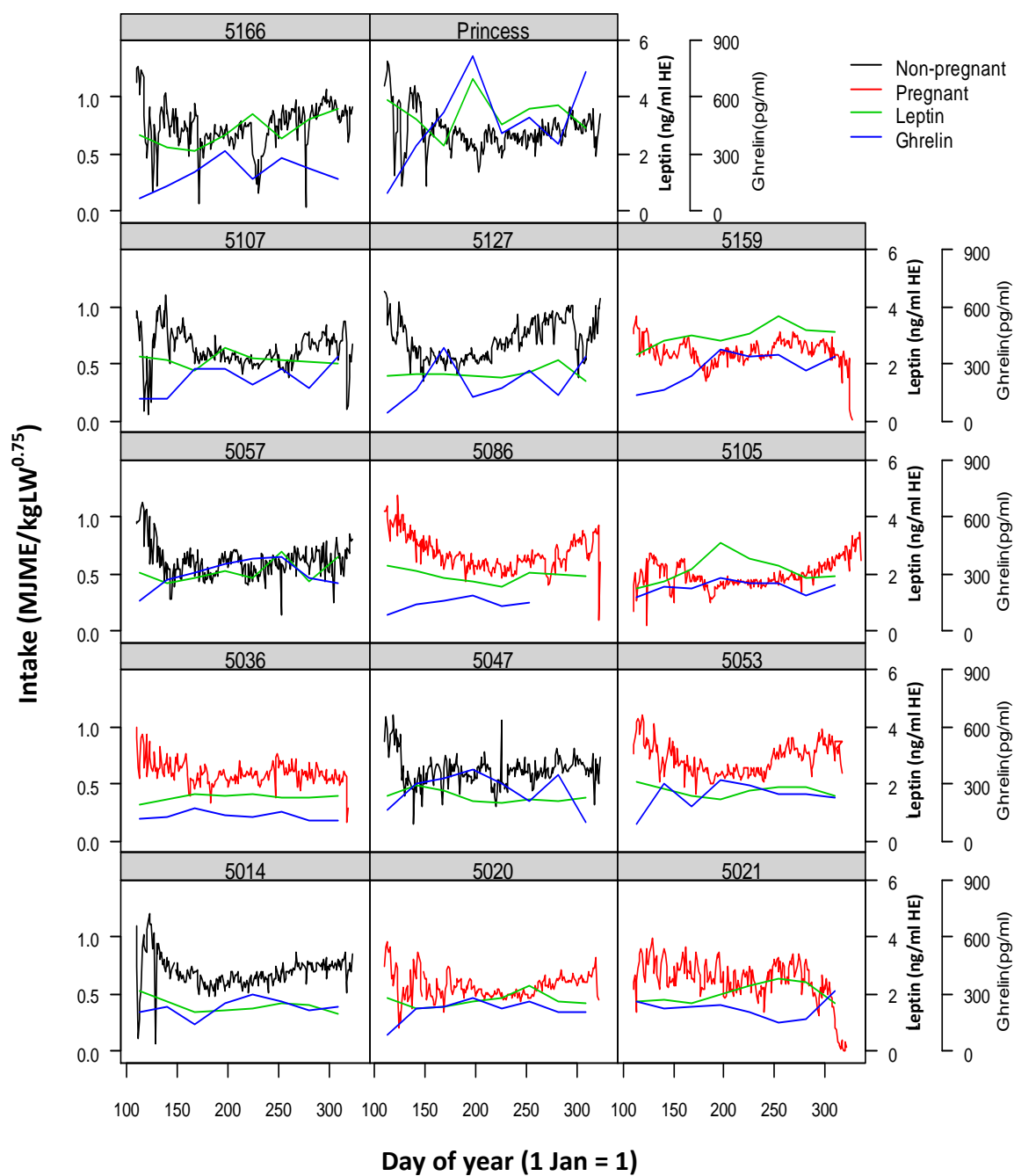


Figure 4.7 Profiles of daily voluntary food intake (MJME/kg LW^{0.75}) of individual pregnant (red) and non-pregnant (black) hinds and their corresponding plasma concentrations of leptin (blue) and ghrelin (green).

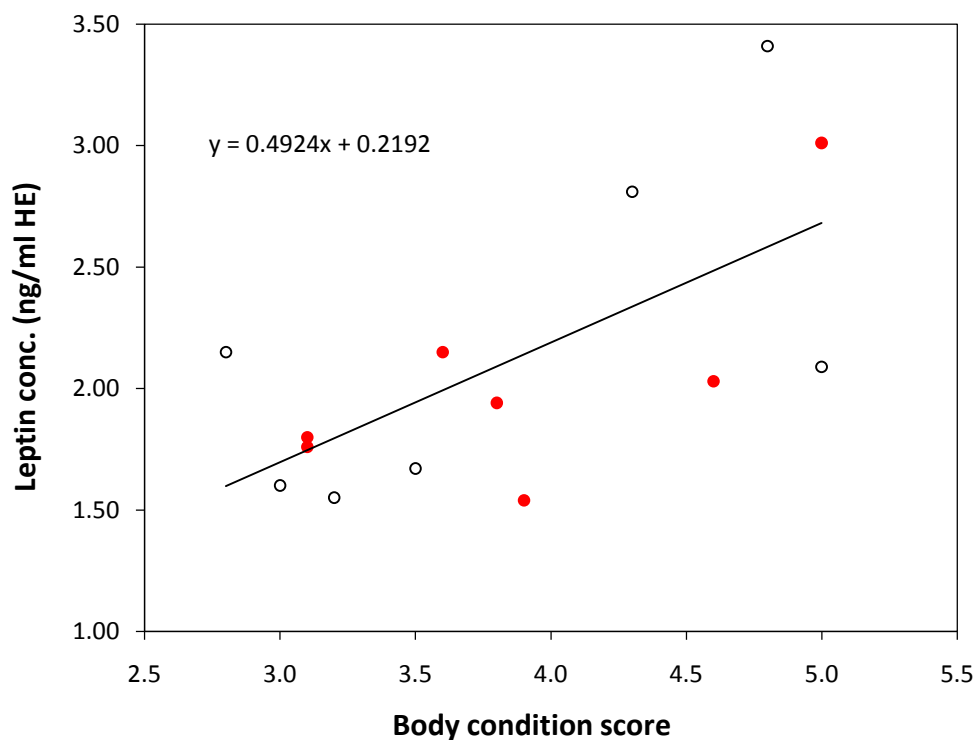


Figure 4.8 Regression of mean body condition score (BCS, 1 = emaciated, 5 = obese) with mean concentration of circulating leptin (ng/ml HE) of pregnant (red) and non-pregnant (black) hinds.

4.5 Ghrelin

Pregnancy status had no significant effect on mean concentration of plasma ghrelin at any of the sampling times (Table 4.6). Mean concentration of circulating ghrelin increased from April to July and then decreased (Figure 4.9), but there was no significant relationship between mean plasma ghrelin concentration and season. Intake of individual hinds was not associated with level of circulating ghrelin. (Figure 4.7).

Table 4.6 Mean concentration of plasma ghrelin (pg/ml \pm SED) at each sampling date. (N.S., not significant)

Sampling date	Pregnant	Non-pregnant	SED	P
23 Apr	136.8	122.8	31.9	N.S.
21 May	204.0	225.1	40.5	N.S.
17 Jun	209.4	309.1	49.0	N.S.
16 Jul	260.4	360.3	87.7	N.S.
13 Aug	225.0	276.0	47.7	N.S.
10 Sep	223.3	308.9	46.0	N.S.
8 Oct	197.7	248.3	38.9	N.S.
5 Nov	222.3	308.1	84.7	N.S.

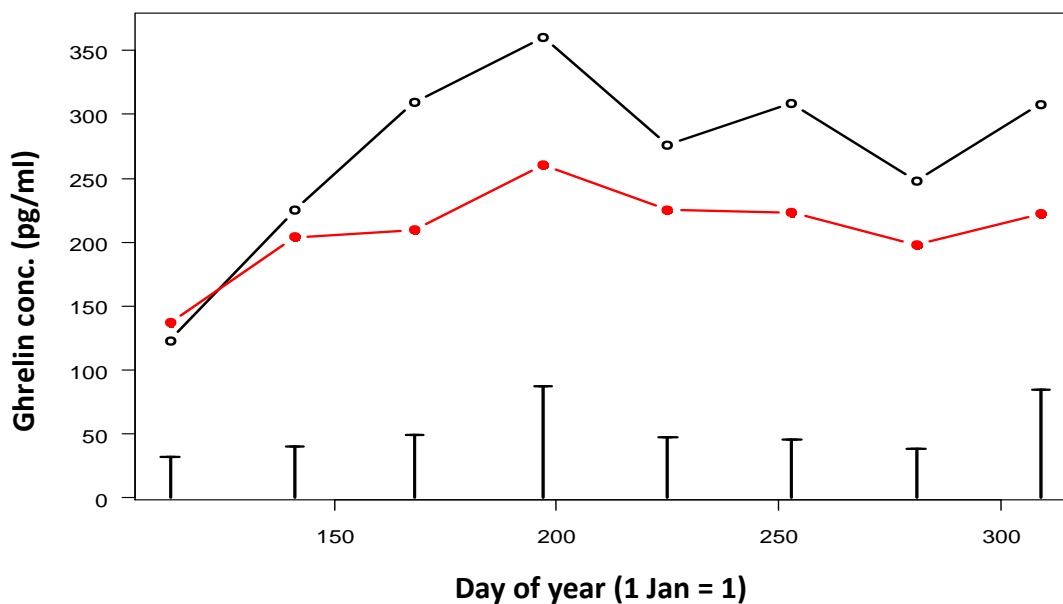


Figure 4.9 Mean plasma ghrelin concentration (pg/ml) of pregnant (red) and non-pregnant (black) hinds. The error bars are the SED at each sampling date.

4.6 Gestation length

Mean gestation length of the hinds was 233.00 ± 2.32 (mean \pm SEM) days with the first hind calving on 13 November and the last on 2 December. There was a significant negative correlation of gestation length with mean VFI during the study period ($r^2 = 0.51$; $P < 0.05$) such that, for every 0.1 MJME/kg LW^{0.75}/day increase in mean VFI, gestation length decreased by 6.4 days (Figure 4.10). Calf birth weight, but not sex, was associated significantly ($r^2 = 0.75$; $P < 0.05$) with gestation length. Gestation length decreased by 4.8 days for every 1 kg increase in calf birth weight.

Gestation length was not correlated significantly with hind live weight and BCS or circulating levels of leptin and ghrelin.

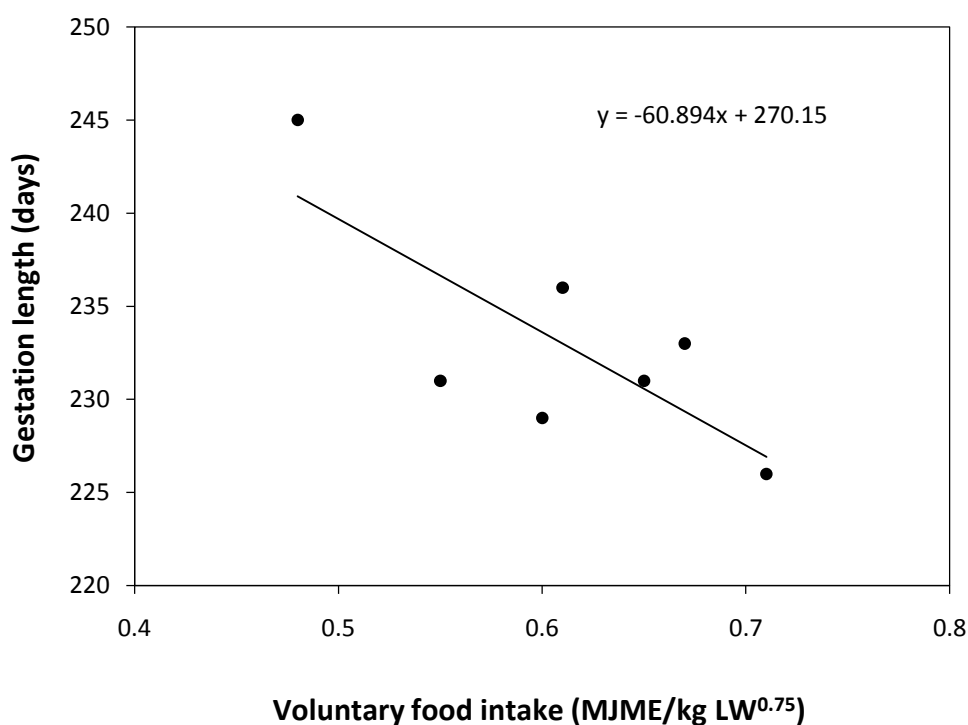


Figure 4.10 Regression of mean voluntary food intake (MJME/kg LW^{0.75}) during the study period with gestation length.

Chapter 5: Discussion

The results from the current study support the hypothesis that pregnancy status of red deer hinds has no significant effect on the, presumed, photoperiod-mediated reduction of VFI during winter. VFI of both P and NP hinds decreased by about 20% from autumn to mid-winter (1 May – 1 July) and then recovered to pre-winter levels by the end of spring (1 November). The amplitude of the change in VFI between autumn, winter and spring in the present study was similar to that reported previously for non-pregnant red deer hinds (Suttie & Simpson, 1985; Loudon *et al.*, 1989). Although seasonal cycles in VFI of housed red deer offered *ad libitum* access to a concentrate diet have been well documented for young growing deer of both sexes, adult stags and non-pregnant adult hinds (see review: Loudon, 1994), this appears to be the first observation reported for pregnant red deer.

Body mass of seasonal animals is maintained within a ‘sliding set-point’ which varies depending on age, reproductive status and season. Hunger is thought to be a consequence of seasonal changes in growth rate, rather than its cause (Kay, 1988). Thus, a seasonally appropriate body mass is maintained through changes in food intake or energy expenditure in response to photoperiod (Adam & Mercer, 2001). Previous studies of housed red deer fed an *ad libitum* pelleted diet have reported that the body weight cycle is associated with that of VFI (Simpson *et al.*, 1983/84; Suttie & Simpson, 1985; Suttie *et al.*, 1987; Loudon *et al.*, 1989; Semiadi *et al.*, 1994; Webster *et al.*, 2000), presumably because change in energy intake is a consequence of seasonal changes in growth rate (Webster *et al.*, 2000). Similarly, in the present study, VFI was lower in winter when LWG was about half of that in spring.

In the present study the overall daily energy intake required to maintain 1 kg of metabolic body weight (E_m) was significantly different between seasons, increasing 1.4-fold from winter to spring. Silver *et al.* (1969) found that the fasting metabolic rate of white-tailed deer increased 1.8-fold between winter and summer, and Holter *et al.* (1976) reported that E_m of young white-tailed deer increased from 0.50 MJME/kg $LW^{0.75}$ /day in winter to 0.75 MJME/kg $LW^{0.75}$ /day (i.e. 1.5-fold) in summer. Simpson *et al.* (1978) reported a smaller increase in E_m requirements of weaned male red deer calves, increasing from below 0.45 MJME/kg $LW^{0.75}$ /day in winter, to 0.50 MJME/kg $LW^{0.75}$ /day in summer. Conversely, Webster *et al.* (2000) found no evidence of

significant variation in E_m between seasons in male red deer calves. The overall E_m of housed weaned male red deer calves fed a concentrate diet was calculated to be 0.45 ± 0.22 MJME/kg $LW^{0.75}$, and the overall energy intake required to increase body weight by 1 kg (E_g) was 53 ± 8.5 MJME/kg LWG. This compares to 0.33 ± 0.21 MJME/kg $LW^{0.75}$ and 90.41 ± 45.21 MJME/kg $LWG^{0.75}$, respectively, in the present study, indicating that adult hinds may require less energy to maintain body weight, but more for LWG, than young stags. However, the small sample sizes and large between-hind variations in VFI and LWG in the present and published studies result in large error values, making it difficult to interpret differences both within and between studies. Furthermore, such estimates must be interpreted with caution because of errors associated with the assumed partitioning of energy intake, especially since seasonal effects on the efficiency of energy utilisation will be confounded with age in the study of Webster *et al.* (2000) and pregnancy status (present study). The latter may be substantiated by there being a significant difference in E_m between P and NP hinds during winter and spring, but not in autumn. The fetus begins to grow rapidly in the last third of pregnancy (Adam *et al.*, 1988a), and thus initiates differences in nutrient partitioning as P hinds ensure the energy requirements of the growing fetus are met (Augustine *et al.*, 2008).

Large variation in both VFI and LWG was a feature of the present study. Some hinds went through cycles of high and low VFI, which resulted in fluctuations in live weight, possibly through variation in gut fill. A diet high in readily available carbohydrates, such as in the present study, may reduce intake due to acidosis (Elam, 1976). Although the ration on offer contained 5% lucerne for roughage to ensure maintenance of rumen function, greedy hinds consuming large quantities of pellets may have suffered from acidosis and reduced their intake, recovered, and then repeated the cycle. Alternatively, those hinds may have been actually regulating their intake to maintain an appropriate 'energy balance' (Scott *et al.*, 2008b). Other hinds consumed a more modest amount of food throughout the study with little variation in live weight. It was noted also that some animals appeared more 'nervous' than others, and from the state of the sawdust bedding, they spent considerable time pacing up and down the pen. Such hinds would expend more energy than others that stayed calm and would therefore require a higher food intake to maintain live weight (Simpson *et al.*, 1978). Also, acute stress leads to increased secretion of corticotrophin-releasing factor, which can cause centrally mediated changes in feeding (Krahn *et al.*, 1984).

Unfortunately, instances of altered hind behaviour were not recorded and it is not possible to substantiate these arguments.

Pregnancy is a dynamic state and to ensure reproductive success the energy demands of the developing fetus must be met at all stages of gestation. In nutritionally poor environments red deer hinds may conceive successfully one year but fail the next because of poor body condition at the time of the rut (Mitchell *et al.*, 1976). During the last third of pregnancy the fetal and maternal components of pregnancy gain about 70% of their final mass in red deer (Adam *et al.*, 1988a), and it was estimated that the additional energy requirements for pregnant, above non-pregnant, hinds increases from 1.7 to 5.0 MJME/day during that time (Adam *et al.*, 1988b). Asher *et al.* (2005a) reported that housed pregnant hinds allowed *ad libitum* access to a concentrate diet increased their daily VFI from 0.6-0.7 MJME/kg LW^{0.75} (~ 20 MJME) at Day 150 of gestation to 0.8-0.9 MJME/kg LW^{0.75} (~ 29 MJME) at Day 210, an increase in daily VFI of ~ 9 MJME, almost double the estimate of Adam *et al.* (1998b). However, the estimate of Adam *et al.* (1998b) was derived from measuring chemical components of the conceptus and makes no allowance for increased body condition of the hinds, as occurred in the study of Asher *et al.* (2005a).

Surprisingly, P hinds in the present study did not increase their VFI in the last third of pregnancy above that of NP hinds during the corresponding period. This is counterintuitive, as one would expect P hinds to require more food than NP hinds, to meet the energy demands of the growing fetus. Between Day 130 (mid-July) and Day 210 (late October) of gestation, P hinds increased their daily VFI by 6.0 MJME and NP hinds increased their daily VFI by 6.9 MJME during the same period. Paradoxically, however, P hinds tended to gain more live weight than NP hinds, although eating less. This may, in part, be explained by the more efficient use of nutrients during pregnancy (Brockway *et al.*, 1963). Moreover, although BCS of non-pregnant hinds increased from 1 September to 9 November, that of pregnant hinds decreased, indicating a moderate energy imbalance during the last trimester of pregnancy. In effect, hind body condition (i.e. fat) served as an energy store and fat was mobilised when VFI was insufficient to meet the energy demands of the fetus. Likewise, pregnant Svalbard reindeer (*Rangifer tarandus platyrhincus*) have large fat reserves in autumn which are used primarily during the last two months of gestation and early lactation, presumably to ensure reproductive success (Tyler, 1987).

Nicol & Brookes (2007) calculated the total energy requirement above maintenance for the entire pregnancy of a red deer hind to be 55 MJME/kg calf birth weight. It is possible to make a similar calculation for P hinds in the present study and compare that value with the amount of energy required for growth of the fetus as calculated by Nicol & Brookes (2007), thus. Mean calf birth weight was 9.8 kg, which would equate to the total energy requirement of P hinds during pregnancy being 539 MJME above maintenance of NP hinds for the same period. Mean E_m and LW of NP hinds during the study was 0.51 MJME/kg LW^{0.75}/day and 132.5 kg, respectively; i.e. total maintenance requirement of a NP hind for 233 days was 4640 MJME. Mean VFI of P hinds during the study was 22.2 MJME/day or 5173 MJME for the mean 233-day gestating period; i.e. 533 MJME above maintenance of NP hinds during the same period. In other words, only 6 MJME less than the 539 MJME estimated for fetal growth according to the calculations of Nicol & Brookes (2007).

While validating the calculations of Nicol & Brookes (2007), such an estimate for the whole gestating period is rather simplistic and does not reflect the true dynamics of energy demand throughout pregnancy. P hinds gained both LW and BCS in the first two thirds of pregnancy, before continuing to gain LW but lose BCS in the final third of pregnancy, when 70% of fetal growth occurs (Adam *et al.*, 1988a). During that time much of the energy for fetal growth came ‘off the back’ of the hinds and highlights the need for hinds to be in good body condition going in to winter. It seems that the observed depression in VFI during winter results in an inability of the hind to meet the demands of a rapidly growing fetus through nutritional intake alone, resulting in an energy shortfall which is met by body reserves during the last third of pregnancy. Consideration must be given also to the fact that hinds in the present study were housed indoors and had access to an *ad libitum* ration of high quality food. Fennessy *et al.* (1981) found that the winter maintenance requirement of stags outdoors was about 50% higher than that for stags indoors, which would exacerbate the negative energy balance under pastoral farming conditions. Hinds in the present study gained body mass throughout winter, but the general pattern of LW change for pregnant red deer at pasture includes a slight weight loss during winter (Fennessy *et al.*, 1981; McNeill *et al.*, 2010).

VFI of the P hinds reached a peak about 3 weeks before parturition then decreased gradually until a precipitous drop in the few days immediately preceding calving. Such a depression in VFI as parturition approaches occurs also in cows and sheep,

and may in these species result in metabolic disorders such as ketosis and hypocalcaemia (Ingvarsen & Andersen, 2000; Melendez *et al.*, 2006). There is a significant negative relationship between the volume of rumen contents and the volume of uterus plus other abdominal organs in sheep (Forbes, 1969). It is possible, therefore, that physical size of the uterus and conceptus in the final stages of pregnancy limits abdominal space available for other organs, thus restricting volume of the rumen (Forbes, 1969). However, hinds in the present study received a high quality diet of pellets containing > 12.5 MJME/kg as compared to a low quality hay diet fed to ewes in the study of Forbes (1969). Therefore, it is unlikely that competition for abdominal space limited VFI in this study. It is worth noting, however, that competition for abdominal space in the final weeks of pregnancy may possibly restrict intake of hinds on low quality feed at pasture. A more plausible explanation for the observed decrease in VFI of P hinds is an effect of the progressive increase in oestrogen levels during the second half of pregnancy, reaching a peak 3 to 0 days before parturition (Tucker, 1985). Intravenous infusions containing quantities of oestrogens similar to those secreted in late pregnancy depressed VFI of castrated male sheep fed a concentrate diet (Forbes, 1971). In addition, corticotrophin-releasing factor (CRF) has been demonstrated to decrease VFI in rodents (Richard, 1998) and sheep (Ruckebusch & Malbert, 1986). Therefore, the CRF-mediated increase in circulating maternal cortisol that is observed in the periparturient period (Tucker, 1985) may also play a role in the precipitous decline in VFI observed at that time in the present study.

Mean plasma leptin concentration varied between 1.84 and 2.35 ng/ml HE in the present study, a range similar to that reported previously by researchers using the multi-species leptin RIA kit on cervid plasma (Suzuki *et al.*, 2004; Soppela *et al.*, 2008; Gaspar-Lopez *et al.*, 2009). Leptin is secreted primarily by white adipose tissue and there is a positive relationship between body fatness and circulating leptin levels in both monogastric (Morgan & Mercer, 2001; Mustonen *et al.*, 2005; Klok *et al.*, 2007) and ruminant (Delavaud *et al.*, 2000; Suzuki *et al.*, 2004) species. Gaspar-López *et al.* (2009) found also that the relationship between leptin and BCS differed with season and plasma testosterone concentration in Iberian red deer (*C. e. hispanicus*) stags, suggesting a role for gonadal steroids in the modulation of leptin secretion. In addition, it has been shown that leptin secretion is modulated by daily photoperiod in ruminants, independently of food intake, body fatness and gonadal

feedback (Bocquier *et al.*, 1998; Soppela *et al.*, 2008). Furthermore, photoperiod has an effect on sensitivity of the hypothalamus to leptin in seasonal mammals, becoming leptin resistant during 'long days' and leptin sensitive during 'short days' (Rousseau *et al.*, 2003; Adam *et al.*, 2006; Zieba *et al.*, 2007). Such a mechanism enables fat deposition during summer and mobilisation during winter as occurred in the P hinds of the present study, but no significant relationship was found between plasma leptin concentration and VFI, stage of season or pregnancy status. There was, as expected, a positive relationship between plasma leptin concentration and BCS, suggesting that level of adiposity plays the dominant role in determining the concentration of leptin circulating in red deer.

Pregnancy has been reported to alter sensitivity of the hypothalamus to leptin in rats, with leptin unable to suppress VFI in pregnant rats, as it does in non-pregnant animals (Grattan *et al.*, 2007; Ladyman *et al.*, 2009). Thus, despite elevated plasma leptin concentration, pregnancy in rats is associated with hyperphagia and increased fat mass. There was no evidence of such leptin resistance during pregnancy in the present study; VFI of pregnant hinds did not increase above that of NP hinds. Moreover, BCS decreased in the last third of pregnancy, as did circulating leptin concentration.

Pregnancy status had no significant effect on plasma ghrelin concentration and there was no significant association between food intake and level of circulating ghrelin. However, it is noted that there was a trend for both food intake and ghrelin concentration to be lower in P than NP hinds from June until the end of the study. Ghrelin is thought to play a minor role in modulating long-term seasonal body weight cycles, but acts predominantly as a short-term regulator of feeding by playing a pivotal role in the initiation of feeding. This role has been firmly established in monogastric species (Wren *et al.*, 2000; Tschöp *et al.*, 2000; Nakazato *et al.*, 2001), but is less certain in ruminants. In cows, plasma ghrelin concentration decreased 1 h after feeding before recovering to pre-feeding levels (Hayashida *et al.*, 2001). In sheep, it has been shown to increase immediately prior to, as compared to an hour before, a scheduled meal and then decline rapidly during feeding (Sugino *et al.*, 2002). However, changing the feeding pattern modified time of ghrelin increase and the authors considered that the observed increases may have been mediated by a conditioned emotional response, rather than hunger. Harrison *et al.* (2008) injected ghrelin through intracerebroventricular cannulae into oestradiol-implanted castrated

male sheep in 'long-day' (LD) or 'short- day' (SD) photoperiods. They found that ghrelin increased VFI in LD but not SD. A similar response has been reported in Siberian hamsters, a seasonal rodent (Bradley *et al.*, 2010). In contrast, Iqbal *et al.* (2006) reported that neither intracerebroventricular nor intravenous ghrelin injection stimulated VFI in ovariectomised ewes, and concluded that ghrelin does not play a significant role in regulating ingestive behaviour in that species. However, the study was undertaken during the breeding season when day length was shortening, and with regard to the study of Harrison *et al.* (2008), ewes may have become refractory to the VFI stimulatory effects of ghrelin.

In the present study, hinds were allowed *ad libitum* access to food and water, and the blood sampling regimen began at the same time on each occasion (1330 h). However, food rations were changed at about the same time each day (0830 – 1030 h) and hinds invariably began eating as soon as they were returned to their pen. It is possible, therefore, that blood sampling took place at the nadir of ghrelin secretion, masking a possible difference in hunger between P and NP hinds. There was no significant effect of season on circulating levels of ghrelin. This is in agreement with Harrison *et al.* (2008) who found that mean levels of circulating endogenous ghrelin were not different between LD and SD. However, the possibility that the present samples were obtained at the nadir of daily ghrelin concentrations means that any seasonal effects may have been masked by the time of sampling.

There was a 19-day spread in calving date despite all hinds conceiving to artificial insemination on 1 April and having *ad libitum* access to high quality food. Asher *et al.* (2005a) reported a negative correlation between duration of pregnancy and change in hind live weight during late pregnancy in hinds on differing planes of nutrition. They hypothesised that fetal induction of parturition is dependent on attainment of a critical size, ensuring birth of a viable neonate. In the present study, gestation length was negatively correlated with energy intake and heavier calves had a shorter gestating period than lighter calves. This supports the hypothesis that variation in gestation length compensates for variation in fetal growth under conditions of a moderate maternal energy imbalance (Asher *et al.*, 2005a). It is interesting to note that 6 out of 7 calves had a birth weight (BW) in the range 9.5-10.5 kg; the remaining calf had a BW of only 7.0 kg after 245 days gestation. The hind giving birth to this calf consumed approximately 0.2 MJME/ kg LW^{0.75} less than contemporaries for much of the study and entered winter (8 June) with a BCS 1.1 unit less than the average for P

hinds on that date. It would appear that in the face of a more severe energy imbalance, prolonged gestation length was unable to compensate fully for the reduced fetal growth trajectory. In this instance, the calf was born at a lower birth weight, as has been reported previously for red deer (*C. e. scoticus*) on the Isle of Rhum, Scotland (Albon *et al.*, 1983), North American wapiti (Thorne *et al.*, 1976) and red deer gestating wapiti (*C. e. roosevelti*) x red deer calves (Asher *et al.*, 2005b).

This study supports the hypothesis that pregnancy status has no significant effect on the photoperiod-mediated depression in VFI of red deer hinds during winter. It is inferred that pregnant hinds are unable to overcome an endogenous cycle of VFI and therefore cannot increase their VFI to meet the energy demands of a rapidly growing fetus in the last third of gestation. Instead, the extra energy required to support the pregnancy is attained through mobilisation of the hinds' body energy reserves, i.e. fat. This highlights the importance of ensuring that pregnant hinds are in good body condition going in to winter, and that high quality feed is available throughout gestation.

Chapter 6: Conclusion

This thesis reports on a study designed to test the hypothesis that pregnancy status has no effect on the photoperiod-mediated decline in VFI during winter.

There was no significant difference between P and NP hinds in mean VFI throughout the study except for the last five days before parturition when VFI of P hinds plummeted, strongly supporting the stated hypothesis. Apparently, this has not been reported previously in red deer. Intake was significantly lower in winter than autumn or spring, seemingly aligned with day length. However, the study was not designed to test if VFI was modulated by photoperiod, therefore, it can only be stated that VFI of both P and NP red deer hinds was lower in winter, than in autumn and spring.

Except for autumn, LWG of both groups was similar, winter LWG being about half that in spring. However, BCS of P hinds declined, whereas that of NP hinds continued to increase, during spring, indicating mobilisation of fat to meet the energy requirements of the rapidly growing fetus. It is hard to conceptualise the evolutionary advantage of a mechanism whereby an animal will lose body condition to support fetal growth when food is available in abundance. However, it must be remembered that deer evolved in the northern latitudes of Europe, where seasonal variation in climate and food availability are more extreme; from abundance in summer, to scarcity in winter. The endogenous cycle of body growth and VFI is thought to have evolved to parallel that of the seasons so that less energy is expended on foraging for food during times of scarcity. As in the present study, pregnant Svalbard reindeer accumulate fat reserves over the summer which are then mobilised primarily during the last two months of gestation and early lactation to ensure reproductive success. The female of the species has thus evolved a fat-biased metabolism to cope with the demands of producing offspring in a harsh environment.

A secondary objective of the study was to measure at different stages of the season the concentrations of two known appetite regulating hormones, leptin and ghrelin, circulating in the body. It was considered that plasma concentration of these hormones may be indicative of the energy status of the hinds, and indeed, the concentration of plasma leptin was associated with BCS, as expected. However, no significant difference between P and NP hinds in circulating concentrations of either

leptin or ghrelin was detected at any of the sampling times during the study, despite P hinds having a negative energy balance in the last third of pregnancy. This indicates that red deer hinds may have an altered sensitivity of the hypothalamus to these hormones during pregnancy, as has been reported for leptin in other seasonal mammals. Although this study was not designed to test such a hypothesis, it would appear that VFI of red deer hinds may be modulated by a change in sensitivity of the hypothalamus to leptin and ghrelin, rather than any variation in concentrations of these hormones circulating in the body.

The design of this study was appropriate to test the stated hypothesis and new knowledge on VFI of pregnant red deer has been obtained. A power analysis based on data from a previous study indicated 6-8 animals per group would be sufficient to detect with 80% confidence a difference in VFI between seasons at the 5% level of significance. Indeed, the present study detected a significant difference in VFI between autumn and winter ($P < 0.05$) and winter and spring ($P < 0.01$). However, conclusions drawn from the study may have been more robust if more animals were included in the study. For instance, a retrospective power analysis of actual data from the study indicated that 24 animals per group would be required to detect with 80% confidence a significant difference ($P < 0.05$) between P and NP hinds in mean VFI during spring. However, such a large number of animals would probably preclude individual penning of animals and would therefore require some sort of automated feeding station to accurately determine individual animal intakes in a group situation. Unfortunately, this would introduce the possibility of behavioural problems due to the hierarchical nature of red deer. It is difficult to recommend a practical solution to the conundrum of having sufficient animal numbers to detect a significant difference between treatments, and the study being practical from a management perspective. In the present study more uniformity in animal history (habituated to handling, reared a calf) and initial LW (94.5 - 168.0 kg) and BCS (2.5 – 4.5) may have helped reduce some of the large standard errors observed, enabling seemingly large differences between mean values to reach significance. However, it was considered ethically responsible to include in the study the eight animals that had, in previous studies, become habituated to handling. Unfortunately a limited number of animals of the same F1 (E x W) genotype were available from the farm to make up the required number of hinds. Nonetheless, including hind history as a variable in the VFI model did not reach significance.

From the results of this study a mechanism of how pregnant hinds meet the energy demands of pregnancy in the face of reduced VFI during winter and early spring has been inferred, and a recommendation on how to manage pregnant hinds to mitigate these effects has been made. Further research to elucidate the inference that hinds are unable to overcome an endogenous cycle of VFI to meet the demands of pregnancy would require using an artificial LD photoperiod during winter/early spring. If the inference is correct, pregnant hinds on such a regime would eat more and produce heavier calves and/or have a shorter gestating period than those exposed to natural day length.

The present study was the first step in testing the hypothesis that early-conceiving hinds have a longer gestating period than those conceiving later because of variation in VFI and fetal growth trajectory. A 2 x 2 factorial study is in progress as the second stage of testing that hypothesis (I.C. Scott, unpublished). Hinds were artificially inseminated mid-March or late-April, housed inside and fed an *ad libitum* or maintenance ration of concentrated pellets; conceptus volume was measured by computer tomography (CT) scan at 120, 150, 180 and 210 days of pregnancy. If the hypothesis is correct, fetal growth trajectory and gestation length will be similar in both groups of early-conceiving hinds and late-conceiving hinds on the maintenance ration, but the fetus will grow quicker, and gestation length will be shorter, in late-conceiving hinds on the *ad libitum* ration.

In conclusion, this study was undertaken with the intention of providing New Zealand lowland deer farmers with a management tool to mitigate the effects of evolutionary adaptation of red deer to climatic conditions in the northern latitudes of Europe. Earlier calving (mid-October) better aligns feed availability with nutritional demand of the lactating hind under New Zealand lowland pastoral conditions, enabling the calf to express its maximum genetic growth potential. To achieve the New Zealand Deer Industry goal of “more, heavier and earlier”, farmers must ensure early-conceiving hinds are in good body condition going in to winter and that high quality feed is available throughout gestation.

Chapter 7: Acknowledgements

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